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DISTRIBUTION OF HETEROCHROMATIN IN THE CHROMOSOMES OF *DROSOPHILA PALLIDIPENNIS*

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STUDIES on the comparative chromosome morphology in species of Drosophila were initiated by Metz (1914, 1923 and other work) and continued by several other investigators. The excellent work of Wharton (1943) gives semi-diagrammatic drawings and descriptions of metaphase chromosome groups in 86 species of Drosophila, together with statements as to the number of chromosome strands found in the salivary gland cells of all these species. The information available permits certain conclusions to be drawn and certain new problems to be formulated regarding the kinds of evolutionary changes in the chromosome structures that have taken place in the phylogeny of this genus. The commonest type of change is inversion of chromosome sections which do not include the locus of the centromere. This could be expected on theoretical grounds, since heterozygosis for such inversions as a rule causes no reduction of the fertility of the flies. The next most frequent type of change seem to be translocations which lead to junction or separation of whole chromosome arms, from the centromere to the free end. Data on the fertility of translocation heterozygotes of this sort are not available and are urgently needed. Inversions of chromosome sections including the centromeres are less common than other changes; translocations which entail exchanges of parts of chromosome

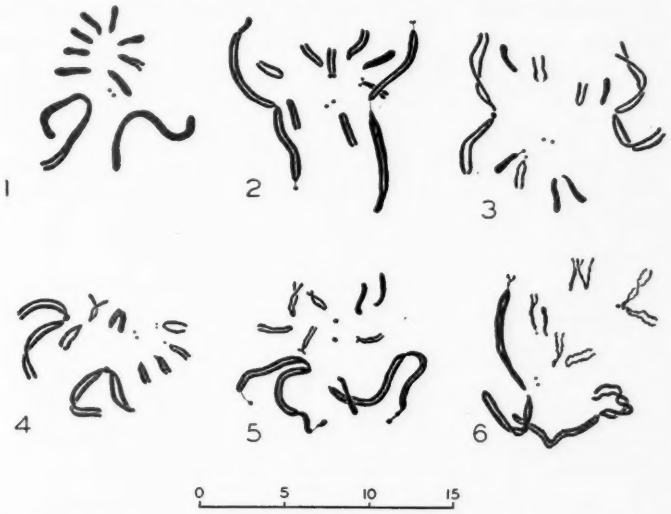
arms are rare, although they undoubtedly do occur. This is because heterozygosis for such inversions and translocations does, judging from examples induced in laboratories chiefly by means of x-ray, reduce the fertility of the flies to a considerable extent; such changes should be discriminated against by natural selection from the time they appear in the populations until they become so frequent that many inversion and translocation homozygotes are produced. The fact that chromosome changes which are supposed to be temporarily deleterious to their carriers may nevertheless become established in some natural populations is very important, since it suggests that under certain circumstances evolutionary alterations may take place notwithstanding the counteraction of natural selection. Because of this, the field of comparative chromosome morphology of Drosophila can not be regarded as exhausted. It would seem, however, that from now on a somewhat more detailed sort of study of at least some selected species ought to be undertaken, to include careful comparisons of the morphology of somatic metaphase chromosomes as well as of the corresponding prophases with the salivary gland chromosome structures. The distribution of the euchromatic and heterochromatic sections in chromosomes and differences in the phyletic behavior of these diverging types of chromosomal materials have been somewhat neglected, although they might shed new light on some important issues.

This article is devoted to a description of the chromosomes of *Drosophila pallidipennis* Dobzhansky and Pavan, which show several interesting and unusual characteristics. *D. pallidipennis* is a species so far known from only a single locality, Furnas, near Iporanga, state of São Paulo, Brazil. It is apparently related to *D. hyalipennis* Duda, described from a single museum specimen from Cuzco, Peru. Both species have a pair of so-called prescutellar bristles, which places them in Duda's subgenus or genus Paradrosophila. Paradrosophila is, however, not a natural group, and the species under discus-

sion should be placed in the genus and subgenus Drosophila s. str., within which they do not however show clear affinities to any other known species groups.

Metaphase Chromosomes

Metaphases in the giant nerve cells (neuroblasts) of larval ganglia have been examined in acetic orcein and acetic carmine smear preparations. In either sex, the



Figs. 1–6. Metaphases and prometaphases in the giant nerve cells of larval ganglia of *Drosophila pallidipennis*. The scale represents 15 micra.

chromosome complement consists of twelve chromosomes, including four pairs of rods, a pair of very large V's and a pair of small dots (Figs. 1–5). In most cells, one of the rod-like pairs is distinguishable from the other three by a slightly greater length. Among the shorter rods, one pair shows in some cells small satellites well separated from the body of the chromosome (Figs. 2–5). This chromosome probably has a subterminal centromere, while in the other rods the centromere appears to be terminal. The dot-like chromosomes are so small that in many cells they can not be identified. The V-shaped elements, which are the X- and Y-chromosomes, are relatively enor-

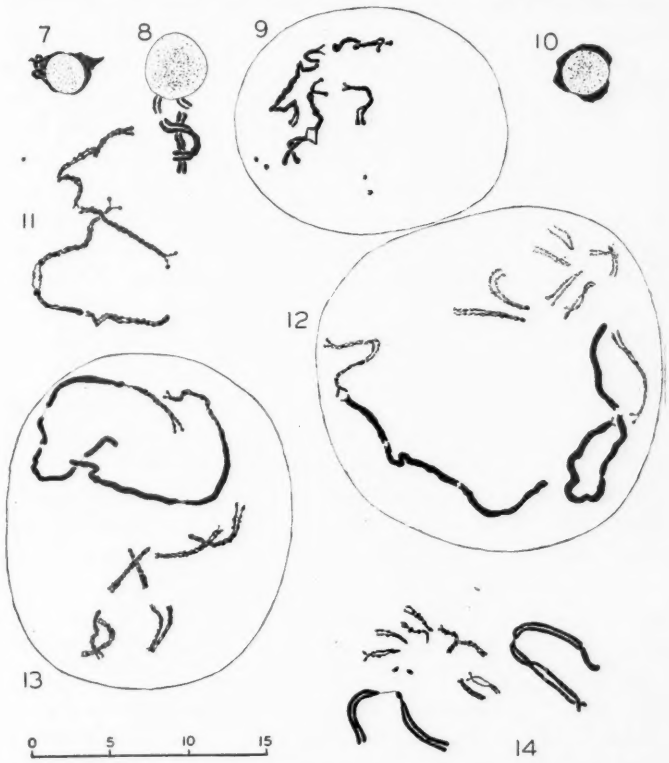
mous. The V's are not equal-armed; the length of the longer arm is approximately equal to the combined lengths of three rod-like chromosomes in the same cell, while the shorter arm is between two and three times longer than the shorter rods. Thus, one of the chromosomes is in the species under discussion longer than all other chromosomes of the haploid set combined. Although great size differences between chromosomes of a species are a common phenomenon, a difference so striking as observed here is not, to the writer's knowledge, encountered in any other animal or plant species. In some cells, especially at pre-metaphase, one or both arms of the V-shaped chromosomes show fairly large terminal satellites (Figs. 2 and 5). The equational splits in the chromosomes are very clearly visible; in fact, in some cells the equational halves of some of the rod-shaped elements lie so far apart that the connection between them at the centromere is invisible (Fig. 3). Such half-chromosomes can be distinguished from whole chromosomes by their smaller thickness. In the V-shaped chromosomes, a secondary constriction close to the centromere is sometimes visible in the shorter arm (Figs. 3 and 4).

Owing to the striking differences in size between the chromosomes, their relative positions in metaphase plates are interesting. The center of the plate is usually occupied by the small dots, with the rods arranged in a circle or a semicircle around them. The large V's lie either next to each other on one side of the plate (Figs. 1, 4, 5) or opposite each other (Figs. 2 and 3). In no cells was one of the V's seen lying inside the other, as V-shaped chromosomes of Drosophila usually do on account of the somatic pairing. The somatic pairing is weak in giant nerve cells, but the smaller brain cells, which have a pronounced somatic pairing, show the same distribution of the chromosomes in metaphase plates as do the large cells.

MITOTIC PROPHASES

The interkinetic nuclei contain a nucleolus, which, in acetic orcein and carmine preparations, appears as an

irregular sphere with a dark outer envelope and a distinctly lighter core (Fig. 10). Some nucleoli show the outer envelope to consist of strongly staining and irregularly convoluted strands (Fig. 7). It is possible that the condition represented in Fig. 7 must be regarded as the



FIGS. 7–14. Nucleoli of resting cells and the prophases in the giant nerve cells of larval ganglia of *Drosophila pallidipennis*. The scale represents 15 micra. The nuclear membrane is omitted in Figs. 7, 8, 10, 11 and 14.

first sign of the onset of prophase. In any case, this condition is met with less frequently than that shown in Fig. 10. The chromatic portion of the nucleolus consists of parts of X- and Y-chromosomes. The association of nucleoli in resting cells with heterochromatic portions of

chromosomes has been described in several species of Drosophila by Heitz (1933a, b), Kaufmann (1934) and others. In *D. pseudoobscura* the nucleolus has one or two satellites around which the condensation of the X- and Y-chromosomes begins (Dobzhansky, 1934). Among the species with which the writer is familiar, *D. pallidipennis* has the largest chromosomal component in the nucleolus.

At the beginning of the prophase, the light core of the nucleolus (the nucleolus proper) becomes separated from the chromosomal parts, which increase in size and begin to show the equational splits (Fig. 8). Immediately thereafter the nucleolus disappears from view. Stages like that shown in Fig. 8 are seen rather seldom. The heterochromosomes rapidly elongate, and the nuclear cavity shows irregularly twisted, intensely chromatic strands with varying numbers of constrictions so strong that the chromosomes appear broken into fragments (Fig. 9). Some of these constrictions probably mark the loci of the centromeres and others the nucleolus organizers, but the writer has not succeeded in distinguishing them. One or two pairs of small chromatic dots may be seen in some, but by no means in all, nuclei (Fig. 9). These dots possibly represent the pair of dot-like autosomes and the satellites of one of the rod-shaped pairs (see above), but this is not certain. The heterochromosomes elongate further and become thinner, leading to a very interesting stage at which the nucleus contains two very long chromosomes with irregular outlines and not a trace of any other chromosomes, except for the above-mentioned small dots (Fig. 11; in this and in Figs. 7, 8 and 10 the nuclear membrane is omitted). A careful examination usually discloses that the irregularity of the outlines is caused by the chromosome strands being composed of alternating thicker and more slender parts, which must be described as chromomeres. This is especially clear in those portions of the chromosomes in which the equational halves diverge more widely. It is, however, not possible to count these chromomeres. The con-

strictions are sometimes more and sometimes less clearly pronounced. The ends of the chromosomes are sometimes elongated to form small pycnotic bulbs, or else are split into paired satellite-like structures (Fig. 11). The precocious nucleination of the heterochromatic portions of chromosomes compared to the euchromatic ones has been described in other species of Drosophila (see Heitz and Kaufmann, *l.c.*), but nowhere is the difference as great as in *D. pallidipennis,* where the heterochromatin is clearly visible while no trace of the euchromatic chromosomes has appeared.

Progressive nucleination of the heterochromatic portions of the X- and Y-chromosomes leads to disappearance of the chromomeric structure and of the irregularity of the outlines (Fig. 12). At the same time there appear very faintly staining euchromatic strands. In favorable cells the number of these euchromatic strands may be counted as nine or ten (Fig. 12). Eight of them are obviously the rod-shaped autosomes, with the equational splits as a rule very clearly visible. One or two of the euchromatic strands are the euchromatic portions of the X-chromosomes. Only one limb of the X has a euchromatic portion, the other limb is entirely heterochromatic. Although the euchromatic and the heterochromatic portions of the X-chromosomes are separated by a large non-staining gap (a secondary constriction), the continuity between them may be clearly indicated (the X-chromosome on the left in Fig. 12). But frequently the gap is so wide that the connection between the two portions can only be inferred (the X-chromosome on the right in the same figure). The gap-like constrictions in the heterochromatic portions continue to be visible, but their number is variable, even in the two chromosomes in the same nucleus. Since, even in the smear preparations, the chromosomes at this stage lie in three dimensions, it is difficult to make exact comparisons of the relative lengths of the euchromatic and heterochromatic portions. The euchromatic parts of the X-chromosomes are clearly much

shorter than their heterochromatic ones, and usually appear to be as long as or longer than any of the autosomes. The total length of the heterochromatic segments is about equal to, and in any case not much shorter than, the total length of all the euchromatic segments. Furthermore, the heterochromatin, as clearly seen in the figures, is concentrated entirely in the X- and Y-chromosomes, with the possible exception of the small dots and of the centromere ends of one of the autosomes, which can be discerned in some nuclei but not in others. Both the large amount of heterochromatin and its complete or nearly complete restriction to the X- and Y-chromosomes set *D. pallidipennis* apart from any of its congeners studied in this respect. The X- and the Y-chromosome may be distinguished because the latter has no euchromatic section. However, the large gaps that frequently exist between the hetero- and euchromatic sections of the X make it easily confused with a Y-chromosome.

The progress of the prophase leads to a nucleination of the euchromatic chromosomes and sections, which now stain only a little less than the heterochromatic sections of the X and Y, but can be distinguished from the latter by uneven outlines and chromomere-like composition (Fig. 13). Cells of this kind are common and frequently admirably clear. Measurements of the chromosome length are still difficult, but it often appears that at this stage the combined length of the euchromatic portions is somewhat, although not much, greater than that of the heterochromatic ones. Equational splits usually disappear in the heterochromatin, but remain visible in the euchromatin. The chromosomes gradually begin to arrange themselves in an equatorial plate, and it is at this stage that an interesting detail presents itself (Figs. 14 and 6). The euchromatic section of the X-chromosome, which is, of course, attached to the heterochromatic portion, loses its chromomeric structure before the euchromatic chromosomes do so. In some cells the differentiation of the X into hetero- and euchromatin becomes obliterated; in others (Figs. 14 and 5), the euchromatic

sections fail to show the equational split which by now is clear in the heterochromatin. Thus, the rate of nucleination of the euchromatic sections seems to depend on the proximity to the heterochromatic ones. By the time pro-metaphase and metaphase are reached, the distinction between hetero- and euchromatin is eliminated entirely (Figs. 1 to 5).

Salivary Gland Chromosomes

Drosophila pallidipennis is excellent material for studies on salivary gland chromosomes. Acetic orcein preparations have been studied. The cells show five chromosome strands each, the terminal and the basal portions of which are shown in Fig. 15 (composite drawings from several cells). Compared to other species of Drosophila, some unusual traits are observable. First of all, a chromocenter uniting the bases of all chromosomes is seldom formed (only two cells with perfect chromocentra from which all five chromosomes radiate have been seen). As a rule, some or all autosomal strands lie free in the nucleus without contact with a very large chromocentral mass. That mass is an integral part of the X-chromosome, which very frequently lies separate in the nucleus with no other chromosome attached (Figs. 15 and 18). The X-chromosome is clearly distinguishable from the autosomes because it is pale and somewhat thinner in male larvae (Fig. 17). Two, three or all four autosomes are frequently associated by their bases (Fig. 16), and one to four autosomes may have their bases in contact with the heterochromatin of the X. It is not possible to decide whether the lack of a regular association of the bases of all chromosomes obtains in living cells or is an artifact produced by the pressure of the cover slip in smearing. Bauer (1936a) has shown that in species of Drosophila with a normal chromocenter, the pressure of the cover slip may break the chromocenter into sections belonging to individual chromosomes, the contact between one or two euchromatic arms joined to a given portion of the chromocenter being usually preserved. In any case,

the association between the bases of the autosomes and the chromocentral mass which wholly belongs to the X-chromosome is weaker in *D. pallidipennis* than in any other Drosophila species with which the writer is familiar. It must be remembered that in some Diptera other than

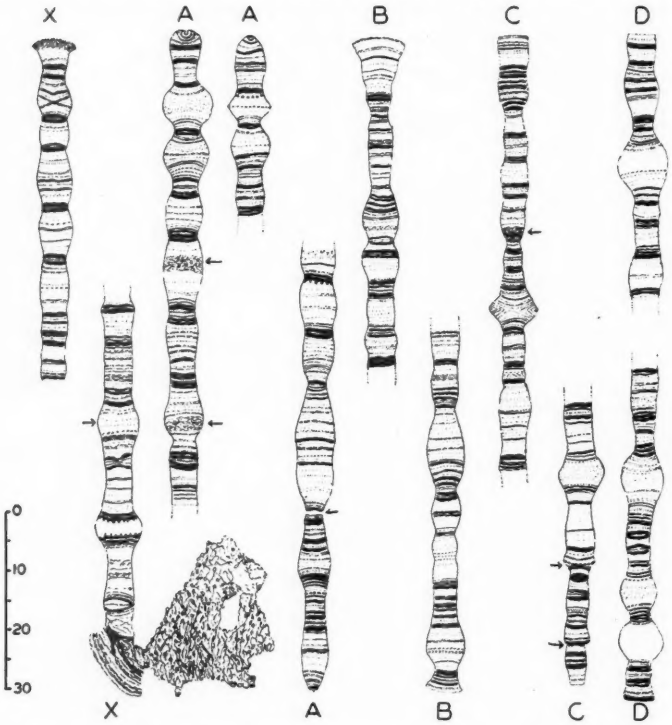


FIG. 15. The distal and proximal parts of the salivary gland chromosomes of *Drosophila pallidipennis*. The scale represents 30 micra.

Drosophila no chromocenter is formed (Metz, 1935, Bauer, 1936, and others).

Examination of the basal portions of the autosomes fails to disclose in them the presence of much material which looks like heterochromatin. This is especially well visible in cells in which the bases of all four autosomes are associated (Fig. 16). Such cells usually show a small heterochromatic mass which, however, is not obviously

associated with any particular chromosome (on the right in Fig. 16), while the bases of the autosomes end in some anastomosing strands which unite the bases with each other and with the small heterochromatic mass. Where that mass comes from is unclear; the most probable guess is that it corresponds to the dot-like autosomes. If so, the latter are composed mostly of heterochromatin. Under these circumstances it is not clear what brings the bases of the autosomes together with one another or with the chromocenter in some cells. We must postulate either a special force, or else the presence of some heterochromatic discs at bases of the autosomes which attract each other, in a manner perhaps analogous to the attraction of the free ends of the chromosomes described by Hinton and Atwood (1941) in *D. melanogaster* and *pseudoobscura*. The free ends of the chromosomes in these species also fail to show clearly heterochromatic discs, and yet they do attract each other. It may be noted that the free chromosome ends in *pallidipennis*, with the exception of the X-chromosome (see below), rather seldom show associations.

The chromosomes are, of course, easily distinguishable by their disc patterns. The longest autosome, labeled A chromosome, has an acuminate free end followed by two inflated bulbs (Fig. 15). The terminal discs are bent inwards to form a funnel-shaped figure (a more and a less extreme expression of this condition are represented side by side in Fig. 15). The same structure of a chromosome end has been described by Bauer (1936a) in a chromosome of *D. hydei*, but whether the chromosomes of the two species are homologous is open to question. Two rather short regions in the distal portion and one in the proximal portion of the A-chromosome (denoted by arrows in Fig. 15) look, in some cells at least, like typical heterochromatin, which is so conspicuously absent at the base of this chromosome. The subbasal heterochromatic section is a "weak place" where the chromosome is constricted and very frequently broken, while the distal sections, on the contrary, tend to flair up and to form inflated bulbs.

Another autosome, B-chromosome, has its free end broadened to various extents in different cells—a condition precisely opposite to that of the free end of A-chromosome. The base of B is also broadened, and in some

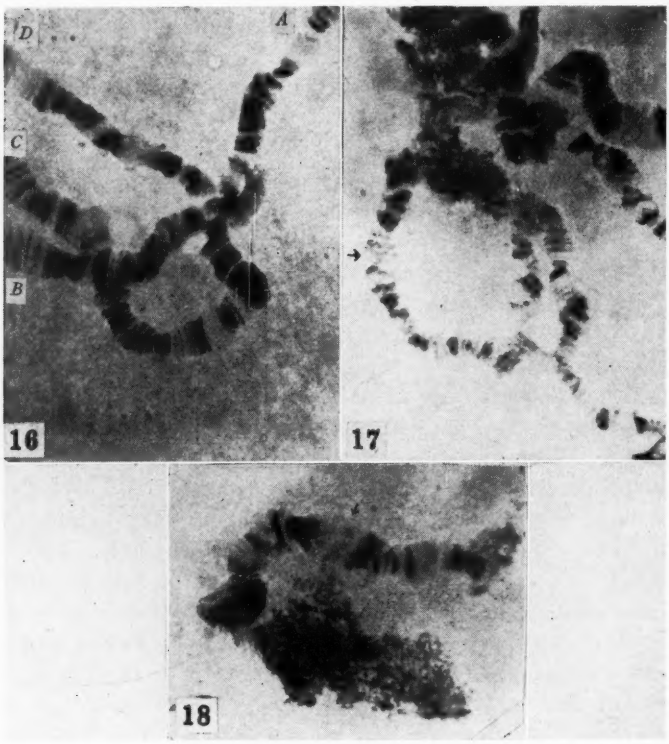


FIG. 16. The basal parts of the autosomes in the salivary gland cells of *Drosophila pallidipennis*. FIGS. 17 and 18. The basal portion of the X-chromosome and the chromocenter in a male (Fig. 17) and a female (Fig. 18).

cells approaches the condition which may be described as intermediate between eu- and heterochromatin. C- and D-chromosomes (autosomes) have more or less cylindrical free ends. In the subterminal portion of C there is a section (marked by an arrow in Fig. 15) which is as typi-

cally heterochromatic as is the chromocenter at the base of the X. Close to the base of C there are two "weak spots" also bounded by what looks like a few heterochromatic discs (arrows in Fig. 15). The short segment of C lying between the base and the most basal "weak spot" is, curiously enough, much thinner in most cells than the rest of the chromosome (this condition is clearly visible in the photograph in Fig. 16). Proximally from the subterminal heterochromatic section, C-chromosome has a bulb which shows in most in most cells a typical "repeat pairing" of the constituent discs; in some cells this condition is diagrammatically clear. The relatively short D-chromosome has, as its distinguishing features, large bulbous inflations at the base and in the subterminal portion.

The X-chromosome is most interesting. The first attempts to draw the disc pattern of its basal portion produced quite divergent drawings. The difficulty proved to be twofold. First, the basal one third to one half of this chromosome contains at least three heterochromatic portions of so considerable an extent that in many cells they are attracted to each other and to the chromocentral mass, and cause the chromosome to be broken and irregularly convoluted (Fig. 17). Second, the limits between hetero- and euchromatin in this chromosome vary so much from cell to cell that a fairly long basal section may in some cells consist of apparently euchromatic discs, while in other cells parts of the same section are included into the chromocentral mass and are no longer identifiable. The base of the X drawn in Fig. 15 is close to the extreme "euchromatic" end of the variation range. Next to the foamy-looking chromocenter proper (comp. Fig. 18) lies a large block of material intermediate between hetero- and euchromatin, which probably represents a large duplication area ("repeat"). This is followed by a short diffuse section, by another small mirror-image duplication, another diffuse section, a "bulb" with some heavily staining discs, a fairly large euchromatic section with two

"bulbs" and a large typically heterochromatic section (not shown in the part drawn in Fig. 15). The photographs (Figs. 17 and 18) also show the basal portions of the X in two different cells (Fig. 17 from a male and Fig. 18 from a female larva). In Fig. 17 three interstitial heterochromatic sections can be seen, two of which are associated with each other and one with the basal chromocentral mass. In Fig. 18 the part of the chromosome lying between the most basal of the interstitial heterochromatic sections and the chromocenter can be seen. A comparison of this part of the chromosome in Figs. 17 and 18 is instructive. The arrows in both figures point at a bulb, which is also marked by an arrow in Fig. 15. In Fig. 18 this bulb is heterochromatin-like and sends a few slender strands to the chromocenter. In Fig. 17 only a short euchromatic section is found between this bulb and the chromocenter. In Fig. 18 the same euchromatic section is seen strongly condensed, but beyond it lies another bulb bounded with heavy discs (compare Fig. 15) and a short apparently euchromatic section which joins the block of the semi-heterochromatic material which probably represents a repeat area (see above). There is, consequently, a fairly large section with discrete discs in Fig. 18 which is included into the chromocenter in Fig. 17. In some cells all of the material up to the heavy discs preceding the bulb marked with arrows in Figs. 15, 17 and 18 is heterochromatic and fails to show discrete discs.

These striking variations in the position of the boundary between the hetero- and euchromatin in the base of the X-chromosome may be compared with the observations of Schultz (1939), who claimed that discs normally lying in the euchromatin change their stainabilities when transferred by chromosomal aberration to the proximity of the heterochromatic chromocenter; in extreme cases these discs disappear entirely. Their loss is used by Schultz to explain certain phenomena of mosaicism in *Drosophila melanogaster*. These observations have been

contested by Sutton (1940), according to whom the euchromatic discs transferred close to the chromocenter retain as a rule their normal properties. The conditions found in the X of *D. pallidipennis* show incontrovertibly that the boundary between the hetero- and euchromatic parts of the chromosome fluctuates so much that many discs which appear euchromatic in some cells are "heterochromatized," become included into the chromocenter, and are no longer identifiable as discrete discs in other cells. There is, however, no need either in this case or in *D. melanogaster* mosaics, to suppose that the genetic materials contained in any of these discs undergo a physical loss. The disappearance of a disc from view may be perfectly well accounted for by Bauer's (1936a, b) hypothesis of the disarrangement of chromomeres in the hetero- compared to the euchromatic regions. This hypothesis is compatible with the assumption made by Schultz (*l.c.*) that the functioning of the genes contained in the chromomeres is altered when the latter are "heterochromatized." This assumption, in turn, necessitates another one, namely, that variable "heterochromatinization" takes place not only in salivary gland cells but in other body cells as well. Direct evidence on this is lacking. Perhaps the observations described above, that the euchromatic portions of the X-chromosome become nucleinated earlier during the mitotic prophase than the euchromatic autosomes, is relevant. The obvious difference between the euchromatic part of the X and the autosomes is that the former has a physical continuity with a large mass of heterochromatin and the latter do not.

The interstitial heterochromatic sections are so large and numerous in the X-chromosome that only its terminal one third may be described as truly euchromatic. Its free end (Fig. 15) shows, however, a structure again resembling heterochromatin. Patterson, Stone and Griffen (1940) draw a rather similar structure in the free end of the X-chromosome of *D. virilis* and Wharton (1942) in *repleta*. Whether this indicates a homology of

the free ends of the X-chromosomes in the three species is uncertain. Similar structures of free ends of chromosomes have also been described in some species of Sciara and Chironomus. In female cells the two X-chromosomes contribute equally to the formation of the huge chromocenter. Two cells have been found in which the X's were accidentally not paired for some distance from the base, and the bases were attached each to a separate half-chromocenter. Since the X- and Y-chromosomes are about equal in size at metaphase, and since the whole Y is heterochromatic, the chromocenter in male larvae must be formed about equally by the X and the Y. Therefore, cells might be found in which the portions of the chromocenter formed by the X and the Y are not fused; such cells have not been found.

The following table shows the relative lengths of the euchromatic parts of the chromosomes, as seen in the salivary gland cells, in percentages of the total length of all the chromosomes in a given nucleus. The data are obtained by measuring the chromosomes with the aid of a measuring wheel in camera lucida drawings of 25 cells selected for having all the chromosomes reasonably well straightened out. The mean values and their standard errors are given.

Chromosome	M ± m
X	18.36 ± 0.47
A	25.60 ± 0.64
B	19.04 ± 0.34
C	21.20 ± 0.65
D	15.76 ± 0.43

A-chromosome is the longest and D is the shortest; the three other chromosomes are about equal in length. The X may be somewhat longer than the data indicate; on account of its interstitial heterochromatic sections the X is seldom as well straightened out as the other chromosomes.

DISCUSSION

The relative amounts of hetero- and euchromatin differ in different species of Drosophila. According to

Sirotina (1938), *D. busckii* has a very large nucleolus but no chromocenter in its salivary gland cells. Nevertheless, the bases of the chromosomes tend to be associated together in this species, as they do in species with clear chromocentra. Since Sirotina has not studied the mitotic prophases, it is possible that in *D. busckii* the chromosomes have heterochromatic parts which do not assume in the salivary gland cell the foamy appearance of a normal chromocenter. The drawings of Heitz (1933a, 1935) show that in *D. virilis* a little less than half of the prophase length of each chromosome is heterochromatin, and yet the chromocenter in this species is small and compact (Heitz, 1934; Patterson, Stone and Griffen, 1940). In *D. melanogaster* only about a fifth or sixth of the prophase chromosome length is heterochromatic (not counting the totally heterochromatic Y-chromosome), but this species has a fairly large chromocenter (Heitz, 1933b; Kaufmann, 1934). *D. pallidipennis* has about half of the total chromosome length heterochromatic, and an enormous chromocenter is formed.

The distribution of the heterochromatic material among the chromosomes is also variable from species to species. The situation which apparently can be regarded as typical (phylogenetically primitive?) for the genus Drosophila is equal apportionment of the heterochromatin among all the chromosomes, except the small dot-like pair and the Y-chromosome, and its concentration chiefly or exclusively near the centromeres. As stated above, the chromosomes of *D. virilis* have each about one half of the length composed of heterochromatin, and they contribute about equally to the formation of the chromocenter in the salivary gland cells. In *D. melanogaster* and *D. simulans* about one third of the X and one sixth or less of each autosomal limb is heterochromatic; the X and the two V-shaped autosomes (corresponding to four limbs) contribute about equally to the formation of the chromocenter. In *D. pseudoobscura,* the X-chromosome is V-shaped, with one of its limbs approximately corresponding to the X and the other limb to one of the auto-

somes of species with a rod-like X; the former limb has considerably more heterochromatin than the latter. Two of the autosomes have each approximately as much heterochromatin as the limb of the X with least heterochromatin, while the third autosome is almost entirely euchromatic; the dot-like autosome is mostly euchromatic (Bauer, 1936, and observations of the writer). Seven types of Y-chromosomes, all of them completely heterochromatic but some more than twice as long as others, are known in different strains of this species. *D. prosaltans* and *D. sturtevanti* have, in their salivary gland cells, about three quarters of the heterochromatin concentrated in two autosomal limbs, while another autosome and the two X-chromosome limbs contain the remaining one quarter (Dobzhansky and Pavan, 1944). The mitotic prophases in these species have not been examined. *D. ananassae* has distributed its heterochromatin still differently: a large part of it became translocated onto what corresponds to the small dot-like autosome in other species of Drosophila, making the latter fairly large at mitosis, while the four autosomal limbs and the X-chromosome each contain a small heterochromatic area (Kaufmann, 1937).

Concentration of the heterochromatin predominantly in a single chromosome is known in some species. *Drosophila hydei* has a V-shaped X-chromosome, one whole limb of which is heterochromatic, while the other limb has a small heterochromatic section at the centromere. The four rod-like autosomes seem to be entirely euchromatic in prophases, or else have only very small heterochromatic sections near the centromeres (Heitz, 1933b). Wholly heterochromatic limbs of V-shaped chromosomes are not rare. Comparing the metaphase chromosome patterns with the corresponding salivaries, Wharton (1943) found heterochromatic chromosome limbs in *D. melanopalpa, fuliginea, mercatorum, hydei, parachrogaster* and *subbadia.* In some other species a rod-like chromosome which is fairly long at metaphase is represented in the salivaries by a small euchromatic section,

evidently because most of its metaphase length is due to heterochromatin. *D. funebris* has five pairs of rod-like chromosomes, one of which, the X, is considerably longer than any of the others. Heitz (1933a) found that about one half of the X is heterochromatic, while the autosomes have only very small heterochromatic sections near the centromeres. In *D. pallidipennis* the amount as well as the distribution of the heterochromatin is unusual. Except for the few interstitial heterochromatic sections in the autosomes, the whole heterochromatin is concentrated in the enormous X- and Y-chromosomes. The centromere ends of the autosomes have few or no heterochromatic discs.

Two mechanisms could bring about the differences in the distribution of heterochromatin among the species of Drosophila. First, heterochromatin may be shifted from chromosome to chromosome by translocations. Second, the amount of heterochromatin within a chromosome may be modified by duplications and deficiencies. Deficiencies take place only rarely in the phylogeny of euchromatic sections, because they affect adversely the viability of homozygotes. But, judging from the relatively little effect produced by losses of heterochromatic Y-chromosomes, it is the quantity rather than the quality of heterochromatin that is important. However, too much reliance should not be placed on the above argument: the heterochromatic sections of chromosomes other than the Y may not be "inert" and may be qualitatively differentiated. If so, the loss of a certain heterochromatic section can not be compensated by reduplication of another section. The similarity of the X- and Y-chromosomes in *D. pallidipennis* suggests that their heterochromatic sections have a common origin, and have been shifted from the Y to the X, or *vice versa,* by crossing-over or translocations. Whether or not both of them had arisen by summation of the heterochromatic sections located in the autosomes of other species of Drosophila is an open question.

Concentration of heterochromatin close to the centromeres is certainly the rule in Drosophila species, although

Kaufmann (1939) and Prokofyeva-Belgovskaya (1939) have presented evidence that some interstitial sections having certain properties of heterochromatin exist in *D. melanogaster*. Concentration of the heterochromatin near the centromeres must, for some reason, be advantageous; this is attested by the fact that none of the rather numerous inversions found in natural populations of Drosophila species involve breaks in the proximal heterochromatic sections, although such breaks are quite common among the x-ray induced chromosomal aberrations. Yet, the rule has exceptions: most of the "euchromatic" part of the X-chromosome of *D. pallidipennis* is really a mixture of hetero- and euchromatic sections. If, as Schultz (1939) believes, transposition of genes normally lying in euchromatin to the vicinity of heterochromatin results in position effects and mosaicism, such transpositions are likely to be discriminated against in natural populations. *D. pallidipennis* shows, however, that the genetic variations so produced need not always be harmful. It should be recalled in this connection that in Chironomus, Sciara and other dipterous genera heterochromatic sections are not necessarily concentrated in any particular portions of the chromosomes (Bauer, 1936b; Poulson and Metz, 1938, and others). Further studies are necessary to clarify these apparently contradictory findings.

SUMMARY

The chromosomes of *Drosophila pallidipennis* are exceptional in several respects. One of its six chromosome pairs is longer at metaphase than all other chromosomes combined. The large chromosomes are X- and Y-chromosomes, they consist chiefly of heterochromatin, and at mitotic prophases they condense long before the euchromatic autosomes and the euchromatic part of the X become visible. In the salivary gland cells, the autosomes (except the small dot-like pair) show no obvious heterochromatin at the centromeres, but some interstitial heterochromatic sections are present. Several fairly

large interstitial heterochromatic sections occur also within the predominantly euchromatic part of the X-chromosome. The boundary between the hetero- and euchromatic sections of the X is variable: many discs that appear euchromatic in some cells are heterochromatic in other cells.

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PHYSIOLOGICAL EFFECTS OF GENES: THE FLIGHT OF DROSOPHILA CONSIDERED IN RELATION TO GENE MUTATIONS

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THE negative survival value of mutant individuals in nature frequently seems much larger than can be accounted for in terms of the obvious morphological differences between the mutant and wild animals. This has led to the rational assumption that single gene mutations generally produce manifold effects. From this point of view, a morphological character differentiating a mutant from the normal wild animal can be neutral in itself and yet the mutation may possess definite survival value as a result of its additional morphological and physiological effects.

An increasing amount of evidence, recently reviewed by Dobzhansky (1941) and Dobzhansky and Holz (1943), has been obtained in support of this theory. For example, it is well established that mutations in Drosophila frequently affect the shape of the spermatheca. It is also generally agreed that mutant animals often show altered and, for the most part, decreased viability (see Dobzhansky, 1941).

Since manifold effects of a physiological nature have an especially direct bearing on the well-being and adaptation of the animal, physiological effects such as those concerning viability are of particular interest. Yet due to the difficulties inherent in quantitative studies of physiological characters, data of this sort are still meager and have generally been difficult to obtain.

In a previous investigation (Reed, Williams and Chadwick, 1942) the frequency of wing-beat during flight was studied on a large number of species and races of Drosophila: under standardized experimental conditions each

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species and race was found to be characterized by a narrow range of wing-beat frequencies. It was therefore possible to separate the various species in terms of this physiological character. Such measurements when performed on suitable hybrid individuals demonstrated that the inheritance and magnitude of wing-beat frequency are conditioned by a multiplicity of genes. This conclusion may seem almost self-evident since the flight physiology involves either directly or indirectly the majority of the physiological and morphological systems of the insect.

From this résumé it is clear that an examination of the frequency of wing-beat of mutant strains should yield some information in regard to the effects on flight of the mutant genes in question. Such an analysis should ideally be performed on mutant strains that differ from the wild "control" strain by a single gene (see Dobzhansky and Holz, 1943), but in practice this objective is very difficult to attain with certainty. In the present report the wing-beat frequencies of an extensive series of approximately isogenic strains of *Drosophila melanogaster* have been catalogued and compared with that of the wild strain from which the former were derived.

Material and Methods

The measurements of wing-beat frequency were performed stroboscopically by means of the technique described by Williams and Chadwick (1943). The animals were fastened to a support and the frequency of wing-beat determined during short flights stimulated at twenty-second intervals by removal of a platform from under the animal's feet. Fifteen measurements were generally made on each individual. All experiments were carried out at $20.0 \pm 0.1°$ C. on virgin female animals 72 ± 2 hours old. The insects were the offspring of single pair matings and were raised under uniform food conditions in a room having a constant temperature of $20.0 \pm 0.5°$ C.

All the stocks of this experiment have the same genetic background. For some years a wild-type stock has been

maintained exclusively by brother-sister inbreeding with a single pair as the parents of each subsequent generation. At the time the animals were tested this strain had reached the 122nd generation of such inbreeding. During the course of this process the strain has undoubtedly changed because of the fixation of new mutations; however, since each generation comes from only two individuals, new mutations must either become fixed or eliminated quite rapidly due to the extremely small effective population size. This method is not as satisfactory for the second and third chromosome as the balancer method; over a long period of time, however, one would expect little difference in the results, unless, due to the continuous occurrence of new small mutations, the balancing is repeated at regular intervals.

Preparations for the present experiment were begun by backcrossing various laboratory mutant strains to the highly inbred wild stock, as indicated in the following diagram. Each generation was derived from a single pair of animals.

1.	♂ mutant	X	♀ wild
2.	♀ heterozygote (from 1)	X	♂ wild
3.	♂ mutant (from 2)	X	♀ wild
4.	♀ heterozygote (from 3)	X	♂ wild
	etc.		

This process was continued for an average of 24 backcrossing generations in order to eliminate the chromosomes derived from the mutant, with the exception of the chromosome containing the mutant gene under consideration. This procedure also served, via crossing-over in alternate generations, to replace all save a short segment of the mutant chromosome with that of the wild strain. Thus the mutant gene to be studied, along with a few closely adjacent genes, was, in effect, introduced into the original wild strain.

Dobzhansky and Holz (1943) have subsequently demonstrated a better method for obtaining isogenism; namely, by x-raying the homozygous wild strain and pro-

ducing the mutations *de novo*. A small number of mutations produced by this method have been studied and will serve as a check on the larger group of approximately isogenic strains established by backcrossing.

Wing-Beat Frequency of the Wild "Control" Strain

Since the effects of the mutant genes upon flight were determined in most cases by a comparison of the wing-beat frequencies of the mutants with that of the wild strain, it is important to estimate as accurately as possible what fraction of any observed differences was due to chance and to external fluctuations and what fraction can be attributed to genetic factors.

As previously described, environmental fluctuations were minimized by flying the animals under rigorously controlled conditions. Nevertheless, due to intangible factors, a certain amount of variability would be expected. The extent of this residual variability was always small, however. As indicated in Tables 1, 2 and 3 the standard errors of the measurements averaged 0.067 thousand wing-beats per minute for the 34 types of animals studied; the average coefficient of variability was 2.87 per cent. Hence it was possible to differentiate the various strains on the basis of even minor differences in their respective frequencies of wing-beat.

Twenty-six specimens of the wild control strain had an average wing-beat frequency of 10.36 ± 0.06 thousand double beats per minute. The reliability of this value has been checked in numerous experiments performed at frequent intervals during the course of the study. It seems justifiable to conclude that if any of the mutant stocks has a wing-beat frequency of less than 10.18 or more than 10.54 thousand double-beats per minute, then a significant effect on its flight has been found.

Mutations Resulting in the Loss of Flight

The following mutants showed complete or extensive loss of flight ability:

maintained exclusively by brother-sister inbreeding with a single pair as the parents of each subsequent generation. At the time the animals were tested this strain had reached the 122nd generation of such inbreeding. During the course of this process the strain has undoubtedly changed because of the fixation of new mutations; however, since each generation comes from only two individuals, new mutations must either become fixed or eliminated quite rapidly due to the extremely small effective population size. This method is not as satisfactory for the second and third chromosome as the balancer method; over a long period of time, however, one would expect little difference in the results, unless, due to the continuous occurrence of new small mutations, the balancing is repeated at regular intervals.

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Mutations Resulting in the Loss of Flight

The following mutants showed complete or extensive loss of flight ability:

Vestigial. The area of the vestigial wings is obviously insufficient to support the animal in the air. Furthermore, wing-motion of this mutant has not been observed. As Harnly (1941) has pointed out, this locus determines the flight capacity of the individual not only through its effects upon the wing form, but also through effects upon the neuro-muscular mechanism, a conclusion with which we concur.

Aristapedia. This mutation results in the loss of the aristae and in striking modifications of the antennae, the segments of which resemble those of legs. Flight and wing-motion are absent, although the wings appear to be unmodified. The failure to produce wing movements can not be directly attributed to the absence of the aristae, since the independent mutant, aristaless, is not flightless.

Bithorax. The degree to which this character reaches expression varies widely even after prolonged backcrossing to the wild strain. When severely expressed, flight ability is lost. However, individuals sometimes move the wings at high frequencies when partially anesthetized. In normal flies the halteres are vibrated at the same frequency as the wings, but in opposite phase: the enormous halteres of this mutant usually remain motionless if the animal can be induced to fly. Two different alleles of bithorax were studied, bx and bx^{34e}; the results were similar for both stocks.

Curved. There seems to be phenotypic variation in the curvature of the wings of this mutant. Otherwise the morphology of the wing appears to be normal. Rare individuals will produce wing movement and support themselves in the air for short distances; usually, however, no wing motions occur and, even in the most favorable cases, the motion is "fluttery" and stroke-amplitude very reduced.

Taxi. The wings of this mutant are apparently normal except for defects in the flexor mechanism that result in the wings being held in the uplifted, open position. No wing-motion has been observed.

Curly. These flies can occasionally be induced to move the wings rapidly, but fail to give any consistent stroke and are unable to support themselves in the air.

Lyra. This mutant is wholly flightless. Morphologically, "scallops" are lost from the two borders of the wing, an effect that is bilaterally symmetrical. The wings are of at least normal length (2.71 ± 0.016 mm), and appear to be unmodified in most respects. The missing scallops reduce the wing-area to 1.64 ± 0.020 sq. mm. from the normal area of about 2.18 sq. mm. One would not, *a priori*, expect this reduction in wing-area wholly to prevent wing-motion. The wings of the mutant, miniature, have an area of but one square millimeter, yet their motion is unimpaired.

Eyeless and cubitus interruptus. About one in twenty of these animals can be induced to fly and support themselves in the air. No animal was found that gave a completely normal stroke and in those cases where wing-beat frequency could be measured it was lower than that of the wild strain. The two individuals on which complete series of determinations were obtained averaged 9.82 thousand double beats per minute, the lowest frequency found for any of the melanogaster stocks.

In summary, it is clear that numerous mutations whose effects are ordinarily considered to be specific structural

changes also produce, or are associated with, marked impairment of the flight ability. Furthermore, the mechanisms of this effect are obscure and frequently have no clear connection with the morphological change by which the mutation is catalogued.

Mutations Not Resulting in the Loss of Flight

The remaining twenty-five mutants showed less extensive changes in flight ability than those described above. The wing-beat frequencies of ten of these mutant strains are recorded in Table 1. As previously described, we

TABLE 1

Wing-beat Frequencies with their Standard Errors, Generations of Backcrossing to the Wild Type, and the Significance of the Deviations from the Wild "Control" Strain

Strain	No. of flies flown	Generations of backcrossing	Wing-beat frequency (1,000 per min.)	Significance
Wild strain (122d generation)	26	..	10.36 ± 0.06	..
Miniature	16	23	12.26 ± 0.11	+
Cut	11	19	10.88 ± 0.04	+
Glass	15	25	10.87 ± 0.11	+
Cinnabar	23	20	10.77 ± 0.08	+
Sepia	25	27	10.33 ± 0.06	−
Bar				
(homozygous)	21	28	10.33 ± 0.08	−
(heterozygous)	22	28	10.44 ± 0.05	−
Lobe				
(homozygous)	21	29	10.11 ± 0.10	+
(heterozygous)	21	29	10.48 ± 0.06	−
Crossveinless				
(homozygous)	24	21	10.15 ± 0.06	+
(heterozygous)	19	21	10.40 ± 0.06	−
Aristaless	13	31	10.18 ± 0.10	−
Scute[1]	20	27	10.14 ± 0.06	+

may expect insignificant variation in wing-beat frequency to fall within the limits 10.18 to 10.54. In Table 1 it is therefore apparent that the mutants, miniature, cut, glass and cinnabar are characterized by significant increase in wing-beat frequency, whereas lobe (homozygous), crossveinless (homozygous) and scute show significant decrease. Sepia and bar fall within the "normal" limits of variation.

The existence of the series of white alleles allowed us to test a single locus more intimately. It is well known that the white locus mutates to varying degrees in terms

of eye pigmentation to give alleles ranging from blood to white. The question naturally arises as to whether the effects of the white locus on flight parallel these gradations of effects on pigmentation.

The results obtained from eleven alleles at the white locus are recorded in Table 2. Significant effects on

TABLE 2

ALLELES AT THE WHITE LOCUS.
WING-BEAT FREQUENCIES WITH THEIR STANDARD ERRORS, GENERATIONS OF BACKCROSSING TO THE WILD TYPE, AND THE SIGNIFICANCE OF THE DEVIATIONS FROM THE WILD "CONTROL" STRAIN. THE ALLELES ARE ARRANGED IN ORDER OF INTENSITY OF PIGMENTATION

Allele	No. of flies flown	Generations of backcrossing	Wing-beat frequency (1,000 per min.)	Significance
Wild strain (122d generation)	26	..	10.36 ± 0.06	..
Blood	21	18	10.77 ± 0.06	+
Eosin	19	25	10.58 ± 0.05	+
Cherry				
(homozygous)	25	24	10.66 ± 0.06	+
(heterozygous)	16	24	10.32 ± 0.06	−
Apricot	33	23	10.40 ± 0.05	−
Honey	21	20	10.56 ± 0.08	+
Buff	21	11	10.87 ± 0.07	+
Tinged	30	24	10.65 ± 0.07	+
Pearl	21	25	10.83 ± 0.06	+
White				
(obtained from Dunn)	23	25	10.45 ± 0.06	−
(obtained from Williams)	19	30	10.42 ± 0.08	−
(obtained from Reed)	21	27	10.79 ± 0.07	+

wing-beat frequency were found for blood, eosin, cherry, honey, buff, tinged, pearl and white (Reed), whereas apricot, white (Dunn) and white (Williams) are without effect. In this table the alleles are arranged in a graded series in terms of pigmentation. It is clear that the degree to which flight was affected is independent of the effects on pigmentation. Apparently some of the alleles at the white locus have effects on flight and other alleles at this locus do not.

X-RAY ISOGENIC STRAINS

Four mutants were studied that had been established by the x-ray method. These consisted of three strains of white and one of apricot. Both homozygotes and heterozygotes were tested in each case. As shown in Table 3 the wing-beat frequencies of none of these strains dif-

fered significantly from that of the wild strain or from that of their respective heterozygotes.

The absence of significant effects in this series confirms the results obtained in the backcrossed strains where apricot as well as two of the three strains of white proved to be without effects on flight. If the backcrossed strains had differed from the wild strain by a large number of genes, such an agreement would not have been anticipated.

TABLE 3

WING-BEAT FREQUENCIES WITH THEIR STANDARD ERRORS AND THE SIGNIFICANCE OF THE DEVIATION FOR FOUR ISOGENIC STRAINS ESTABLISHED BY THE X-RAY METHOD

Strain	No. of flies flown	Wing-beat frequency (1,000 per min.)	Significance
Wild strain (122d generation)	26	10.36 ± 0.06	.....
Apricot			
(homozygous)	17	10.32 ± 0.08	Negative
(heterozygous)	18	10.19 ± 0.07	"
1st White			
(homozygous)	16	10.26 ± 0.08	"
(heterozygous)	21	10.20 ± 0.05	"
2d White			
(homozygous)	27	10.39 ± 0.04	"
(heterozygous)	28	10.31 ± 0.05	"
3d White			
(homozygous)	27	10.58 ± 0.06	"
(heterozygous)	24	10.43 ± 0.06	"

DISCUSSION

The data recorded above reveal that of the 33 homozygous mutant strains studied, 24 per cent. showed complete or extensive loss of flight and 45 per cent. showed significant alterations of wing-beat frequency. In less than a third of the strains was flight unaffected. Of the ten strains in the latter category, seven were at the white locus and five of these were the mutant white itself. Thus the latter appears to be singularly free from effects on flight. In terms of wing-beat frequency these five white strains appear to be identical and perhaps should be grouped together.

There can be little doubt that the observed differences have a genetic basis. Assigning the effect in each case to a particular mutant gene is not justifiable, however, since

the number of residual foreign genes and the extent to which they may have participated is unknown. But a consideration of the total evidence strongly suggests that some mutations by themselves have effects on flight whereas other mutations are without effect.

In the case of the eight strains in which flight was wholly or extensively lost, the effects of these mutant genes appear to be so extensive that the degree of isogenism is probably of minor importance. From a standpoint of the survival of these strains in nature, it is therefore apparent that the loss of flight is of vastly greater significance than the comparatively minor morphological changes usually assigned to these genes.

The present data consequently support the theory of manifold gene effects and illustrate the danger of evolutionary considerations based solely on morphological characters.

SUMMARY

By means of a stroboscopic method of measuring the frequency of wing-beat, thirty-three approximately isogenic mutant strains of *Drosophila melanogaster* were tested for physiological differences, as indicated in the flight response.

Twenty-four per cent. of the mutant strains showed complete or extensive loss of flight. The mechanisms of this effect are obscure and frequently had no clear connection with the morphological changes usually assigned to these genes.

Forty-five per cent. of the mutant strains could fly, but possessed wing-beat frequencies significantly different from that of the wild, "control" strain.

In less than a third of the mutant strains was flight unaffected.

The genetical basis of these effects is discussed and it is concluded that in at least a number of strains the effect may be assigned to the mutant genes. Conversely the absence of effects was demonstrated for a number of other mutants.

The present data therefore support the theory of manifold gene effects and emphasize the danger of evolutionary considerations based solely on morphological characters.

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GENETICS OF BODY SIZE AND RELATED CHARACTERS

II. SATELLITE CHARACTERS ASSOCIATED WITH BODY SIZE IN MICE

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PART I described the formation from one stock by selection of (1) a small-bodied race and (2) a large-bodied race of the common mouse. Though attention during the selections was directed solely to changing body-size itself, yet the races so produced have come to differ markedly in several other respects, for which we did not select them.

The two races have thus become progressively, and often strikingly, differentiated in the frequency with which they exhibit certain hair-color and ear-length genes; in their activity and behavior; in the comparative length of their various appendages (the tail, hind foot and ears, especially); and in litter size (number of young born per litter).

Being wholly incidental to the body size selections in their first appearance and their subsequent development, these changes are to be regarded as secondary or satellite characters, clustering around and correlated with growth in body size as the primary or central character.

In this paper the differences between the races will be briefly described, the nature of the correlating mechanisms analyzed in a preliminary way, certain basic physiological problems pointed out, and a few applications of more general significance made.

A. BEHAVIOR DIFFERENCES BETWEEN THE RACES

Individuals of the small race of mice are very active and aggressive, and rather savage, as compared with the tamer and more docile large race. The small mice are so agile and elusive that their capture from a cage becomes

something of a problem, and they have to be weighed in a container. Their "wildness" is obvious to all who work with them, but no attempt has yet been made to measure or analyze it or to determine its underlying causes. The behavior differences accompanying body size differences may simply be an expression of basic metabolic levels; the large mice do tend to become sluggish and obese.

Domestication is, of course, frequently associated with some increase in both body and litter sizes. In general, the behavior of the mice as they were made larger by selection often recalls the findings of Keeler on the changes of temperament occurring in the breeding of the Wistar rat, and on particular coat colors as "marks of domestication." Fichter and Davis mention further cases of such an association in mammals. It is noteworthy that the changes of temperament are tied up with various colors, which are not necessarily homologous even in rats and mice.

B. Coat Color Differences in the Small and Large Races

It is a matter of some interest, with definite bearing on the nature of size inheritance, that selections made for body size alone, ignoring color entirely, have quite unintentionally altered hair colors in the two divergent races.

The contrasting color types under discussion were all noted in early generations of both S and L stocks, but mice of the small race became preponderantly, and in the end exclusively, intense black, black-and-tan or agouti; they are quite frequently piebald, but apparently never dilute or albino. In them the genes *B* and *D*, and possibly *C*, approach or have actually reached fixation, and *s* persists. In the large race, however, the mice have become solid or self-brown or cinnamon (with an occasional albino), and are often dilute. These facts indicate that the recessive genes *b*, and perhaps *d*, are, or are being, made homozygous, and the piebald factor *s* lost.

Such results had not in fact been anticipated, but can be logically explained along lines of recent genetic research. (1) Various investigators, especially Castle, have reported that genes *b* and *d* characteristically accelerate growth in the mouse, as one of their pleiotropic effects, while *B, D* and *s* (as well as short ear, *se*) retard growth in some degree and make for smaller body size. Selection for larger body size would thus naturally increase frequency of qualitative genes like *b, d* and *S*, while the small race would gradually accumulate and fix the contrasting allels. (2) Since the short ear factor, *se*, is closely linked with dilute, it is not surprising that homozygous short-eared mice, smaller by several grams than their litter mates, still segregate from some pairings of the large race. The pull of *d* in one direction (toward size increase) is greater than that of *se* in the other (size decrease); the cross-over combinations *dSe* or *Dse* will be favored by selection. (3) The occurrence of albinos in the large, but not in the small race, is doubtless the result of chance loss of the *c* allel due to drift in a small breeding population (Wright).

As far as the general divergence in size is concerned, it is easy to emphasize unduly the growth-accelerating or retarding effects of a few qualitative factors. We have handled litters, and undoubtedly could establish stocks, of very large body size which carry all the growth-retarding genes mentioned in (1) above. Selection to increase size would certainly succeed without these particular pleiotropic effects, or in spite of them. This important fact is practically ignored in some recent discussions of size inheritance.

C. Changes in Relative Length of the Appendages Accompanying the Selections for Body Size

The large and small races are to a merely casual inspection normally well proportioned, and measurements of the cranial, axial and appendicular parts have changed somewhat as one would expect considering the changes

in body size. Typical linear measurements have increased in the large and decreased in the small race in the course of the selections to alter body weights. Body length (snout to anus by Sumner's method) of males, for instance, averaged 81.28 mm in S_8 and 96.73 in L_8, a highly significant difference of over 28 standard errors. The lengths of appendages (ears, hind feet and tails) also diverged significantly (Table 1).

This is not to say, however, that all organs and regions of the body have changed at precisely the same rate as the body as a whole. The head itself, and all the various

TABLE 1

COMPARISON OF AVERAGES OF ABSOLUTE MEASUREMENTS (BY SUMNER'S METHOD), AND RELATIVE LENGTHS OF APPENDAGES (TAIL, EAR, HIND FOOT) IN MALES OF THE SMALL AND LARGE RACES OF MICE PRODUCED BY EIGHT GENERATIONS OF SELECTION FOR BODY SIZE (60-DAY WEIGHT) ALONE. THESE APPENDAGES ARE ALL COMPARATIVELY SHORTER IN THE LARGE RACE, WHICH IN THESE RESPECTS RESEMBLES GEOGRAPHIC RACES INHABITING COLD CLIMATES

	Small race (S_8)	Large race (L_8)	Difference / standard errors
Absolute length (mm) of:			
body (BL)	81.28 ± 0.44	96.73 ± 0.31	28.6
tail (TL)	75.78 ± 0.58	85.54 ± 0.46	13.2
ear (EL)	12.60 ± 0.07	13.82 ± 0.05	15.2
foot (FL)	17.12 ± 0.10	19.05 ± 0.06	16.1
Relative length of appendage:			
TL/BL	0.931 ± 0.005	0.882 ± 0.005	7.0
EL/BL	0.159 ± 0.001	0.149 ± 0.001	7.1
FL/BL	0.211 ± 0.001	0.197 ± 0.001	10.0

appendages, are comparatively small in the large race. Owing to slower relative growth, the appendages do not keep pace with growth of the body. Table 1 presents relevant data.

Nature of the correlating mechanism. The remarks we have to make about body and appendage lengths require some short summary of our observations on growth and relative growth (Huxley) in these animals. It is generally appreciated that a fetal mouse is exceedingly short-tailed. At birth the tail is proportionately longer, but is still only about two fifths as long as the body. For a brief period after birth the tail continues to lengthen much faster than does the body itself; this is the period of marked positive heterogony (tachy-heterauxesis) in tail

growth. The result is that by the time the mouse body measures 2½ to 3 inches, the tail has become nearly as long as the body. If the mouse body continues to grow even more, however, to 4 or 5 inches, for instance, the tail growth is found to lag behind by negative heterogony (brady-heterauxesis), so that eventually the tail is again found to be definitely shorter than the body. What has been said about the tail applies also to the foot and ear, except that the timing of growth is somewhat different in the latter organs.

The differences in appendage length in the mouse races can now be understood and explained as a case of allomorphism arising out of ontogenetic heterauxesis (Huxley, Needham and Lerner). The growth rate of the small race is generally retarded; its size increase slows down sharply in the second month, and practically ceases at a body length of at most 85 or 90 mm. At this body size the tail and other appendages are still comparatively long. In view of the relative growth described it may be predicted that further reductions of body weight and length in the small race will result in further increases in relative length of the appendages. Ultimately, however, decreases would again be expected.

Growth in the large race, by contrast, is generally accelerated and possibly more prolonged. The body grows on to a length of 110 or 120 mm. The tail meanwhile fails to keep up with the rate of body growth, and the indices, TL/BL, etc., accordingly fall to lower values than in the small race. These indices are all significantly lower (Table 1).

Measurements of body and appendages taken on large-race mice at frequent intervals from birth onward show that relative lengths of appendages match those in the small race at corresponding body sizes, but that when body size exceeds that of the small race the appendages pass farther into the phase of brady-heterauxesis and are relatively shortened. An immature 20-day large-race mouse resembles quite closely a 60-day mouse of the

small race in size, body proportions and comparative length of appendages.

The present case, discussed at greater length elsewhere (MacArthur and Chiasson), is possibly the first in which size races of recent and known origin have been available to study allometric phenomena both as ontogenetic processes (heterauxesis) and as phylogenetic divergences attained (allomorphism). From their mode of origin by selection, the two races are likely to differ only, or predominantly, in general size or growth-rate factors, and not (except as chance fixation or drift enters in) in growth factors which act regionally or locally, and might specifically control tail, ear or foot lengths. The differences noted in the comparative size of appendages are thus to be attributed with some certainty to general, rather than to any specific local factors.

D. Changes in Litter Size Accompanying Selection for Body Size

We gradually came to realize, with the progress of the selections for body size, that the number of young born in a litter was rising in the large race and falling in the small race. This difference soon became both striking and significant, as the data in Tables 2 and 3 will show.

In mice, as in mammals generally, there is an age factor in liter size (see Hammond; MacArthur, 1942). In both the mouse races the second or third litters contain, on the average, more young than either the first, fourth, or fifth litters.

The important interracial difference is that the small race had litters of from 3 to 8, the large race of from 3 to 15, young, the range and variability being much greater in the L stock.

Some additional data relating litter size to weight of new-born young and weight of mother are given in Table 4. As compared with those of the small race, young from litters of the large race were more numerous by 84 per cent. and individually heavier by 24 per cent. The col-

TABLE 2

BODY SIZES (60-DAY WEIGHTS IN GRAMS) AND LITTER SIZES (YOUNG BORN PER LITTER) IN SMALL AND LARGE RACES OF MICE PRODUCED BY EIGHT GENERATIONS OF MINUS AND PLUS SELECTION, RESPECTIVELY

	Small race (S_8)	Large race (L_8)	Difference / standard error
Body weight (grams)			
Males	16.14 ± 0.25	33.71 ± 0.31	44.1
Females	13.80 ± 0.19	28.43 ± 0.39	33.7
Number of young per litter			
First litter	5.00 ± 0.19	9.22 ± 0.70	5.8
Second litter	6.05 ± 0.32	10.75 ± 0.78	5.6

lective weight of a whole litter in the large race was over twice as much as in the small. Reproduction was equally "expensive" in the two races, if we judge from the fact that in both, the total litter weight is about a third that of the mother just after the birth of the young. But the

TABLE 3

FREQUENCY OF OCCURRENCE OF LITTERS OF 3 TO 15 YOUNG IN THE SMALL (S_8) AND LARGE (L_8) RACES OF MICE

	3	4	5	6	7	8	9	10	11	12	13	14	15
Small race													
first litter	3	5	12	5	3								
second littler	1	3	5	4	3	6							
third litter			3	1	3								
Large race													
first litter	1		1	1	2	4	1	1	1	3	2	1	
second litter		2			1	1	2	2	3	2		1	2

weight of an individual progeny comprises only 3.6 per cent. of the mother's weight in the large race, and about 6.7 per cent. in the small. Delivery of the young is probably an easier process for the large race mother.

The physiological correlation of body size and litter

TABLE 4

NUMBER OF YOUNG AND SIZE OF BIRTH-FED YOUNG IN SUCCESSIVE LITTERS OF THE SMALL (S_8) AND LARGE (L_8) RACES OF MICE, AND THEIR RELATION TO MOTHER'S BODY SIZE AFTER BIRTH OF YOUNG (ALL WEIGHTS IN GRAMS)

	Mean litter size	Mean wt. of birth-fed young	Total wt. of litter	Mother's weight	Litter wt. / mother's wt.
Small race					
first litter	5.0	1.15	5.66	17.06	.33
second litter	6.1	1.22	7.12	19.65	.36
third litter	6.0	1.13	7.10	20.14	.35
Large race					
first litter	9.2	1.49	13.67	41.05	.33
second litter	10.1	1.47	14.92	46.77	.32

size. The facts of this association are clear enough. Why, then, does a small race female have few young, and a large race mother about twice as many in a litter?

Litter size in mammals may be limited and controlled in general by: (a) the number of ova made available by the female, (b) the proportion of these eggs successfully fertilized by the male and (c) the prenatal mortality of zygotes between fertilization and birth of the young. These factors seem to be important in the order named in causing the difference in litter size in the mice. It is hardly tenable as a hypothesis that litter size is primarily equal in both races, but is cut down in the small race by extra mortality to suit the capacity of the small mother. Direct examination of pregnant females and counts (mainly by Mr. L. P. Chiasson) of their corpora lutea and fetuses, show that the two races differ greatly only in number of ova released. Corpora lutea averaged about 7.5 in small, and 13.6 in large race females (of generation 11). The ceiling on egg number, like that on fetuses, or young born, is nearly twice as high in the large race.

On purely anatomical grounds, it might be thought that a large mouse, with proportionately large ovaries, would, of course, liberate ova in numbers corresponding to the mass of her ovarian tissue. In mammals at least the known facts are against this simple explanation, for as many eggs appear to be freed from one ovary, or a half ovary, as from two. Reviews by Hammond, and by Moore, cover this and other phases of the problem of mammalian fertility.

From the general physiological and clinical evidence, the correlation between body size and litter size depends on endocrinal links, such as the growth and gonadotropic hormones of the anterior pituitary, which presumably mediate in some way between the size genes and their diverse ultimate effects. The precise nature of the nexus remains to be determined. Size genes may control primarily the size of the pituitary, and thence, secondarily, both body size (by the growth hormone) and litter size

(by the follicle-stimulating gonadotropic principle). More likely the size genes primarily regulate general body growth, and thence, by laws of relative growth, the size of the pituitary, whose gonadotropic hormone, acting quantitatively, determines the litter size. The number of ova released by a female thus in all probability depends, not upon the size or even the maturity of the ovaries, but on the amount, concentration, activity of the F.S. substance, which either from injections or pituitary transplants easily induces (see, *e.g.*, Rowlands) marked hypertrophy of ovaries and increased output of eggs (superovulation).

In any event, the mouse data suggest that general body size factors exert a remote and indirect control over litter size. There is little reason to think that the two races carry different "litter size genes" or "fertility factors" as such.

DISCUSSION

It is, perhaps, only to be expected, after all, that selections to alter body size should result in coordinated changes in some other quantitative and qualitative characters. Only on the view that there is a single specific gene "for" each character could one expect otherwise. More nearly justified is the contrary view that a gene typically helps determine, and controls grades of expression of, a whole system or syndrome of supposedly unrelated characters and parts.

The characters thus grouped proved to include all kinds: continuous and discontinuous; morphological, physiological and psychological; qualitative (as hair colors), meristic (as number of young) and quantitative (body size, activity, etc.). The correlating mechanisms involved are doubtless equally various, some being causal in nature and permanent in effects (as in the cases of pleiotropic, or physiological correlations) and others merely statistical and temporary (as in case of linkage, *e.g.*, of short ear in the large race of mice). Correlations

due to linkage or to "drift" are likely to be sporadic and erratic; those due to manifold gene effects are likely to be of more regular and widespread occurrence, and susceptible of formulation as more general "rules," like the association of large body size with large litters in races of many species of mammals (MacArthur).

If a gene acts pleiotropically its several effects are not clearly separable. In achieving its diverse end results ontogenetically, a variety of mechanisms and processes, cyto-histological, physiological, endocrinal and nervous, are frequently concerned. Selection for a faster growth rate must provide more cell units in the body as a whole and in its several parts, including the endocrinal glands. This offers a basis for gradations of functional activity in hormones, and these in turn for changes, often more or less regional and local, in physiologically correlated characters. Changes in body size are bound to introduce changes in the relation of body surface to mass, and in metabolic levels and activity. By heterogonic growth they also alter relative length of appendages and perhaps even number of young born in a litter. Such varied correlated characters trace back link by link by one causal chain or another to more general, antecedent growth processes and ultimately to common genes or gene complexes.

Factors controlling a character like body size are undoubtedly numerous enough to be represented in many or all chromosomes and segments of chromosomes. Size genes are quite sure to be in some degree genetically linked with qualitative, multiple, balancing or modifying factors controlling other characters. In this sense all quantitative characters are associated by linkage, as Anderson has shown for several graded characters in a tobacco species cross. Variability in several respects and characters, both quantitative and qualitative, is thus set free simultaneously by new combinations following crossing over. The characters in such a complex are held together, at least briefly, and are changed concurrently by selection directed on any one of them.

Modern genetic theory thus returns, after a long diversion, to the general principle so insistently emphasized by Darwin, that selection produces correlated, rather than a series of independent, changes. Some of the correlated changes described in the mice are obviously adaptive when associated. It is only fitting, for instance, that a large mouse should bear the large litters. But, by way of warning against putting too much trust in pure a priori reasoning about the fitness of things, we should note that the number of nipples for feeding the extra young is not increased in the large race; nipple number is apparently an independent character in mice, as in sheep. Nor is the length of the gestation period noticeably changed by the selections for growth rate.

A group of correlated characters, adaptive enough frequently to become established in nature by natural selection, is seen in the size races of species of warm-blooded animals of wide range and more or less continuous distribution. The geographic races inhabiting colder habitats (high latitudes and altitudes) tend to be those of larger body size (Bergman's rule), and those presenting relatively smaller body surface and smaller appendages (Allen's rule); in both respects they economize on heat lost by radiation. That large-bodied races should also have larger litters (the "litter rule" for mammals) or clutches (Rensch's "clutch rule" for birds) may also be considered adaptive. In any case the characters enumerated in these climate rules are clearly related as a common complex, based directly or indirectly on the same general size factors, arising together in development, and often keeping together in their geographic distribution.

In the course of further raciation and speciation such complexes of associated characters are often worked over and extensively reorganized. Between allied species of mice of the genus *Peromyscus* Clark has found that differences in "body size and proportions cannot be attributed primarily to general size factors and heterogonic growth," the variations in tail, body and foot lengths,

for instance, being in this case "due primarily to special size factors, genetic and developmental."

Applications

It should be of some interest to field naturalists and museum biologists making faunal surveys that so many "climate rules" or trends, originally formulated and established as quite independent of each other (see Rensch), prove to have a common underlying basis.

It is rather remarkable that two races of mice, bred from one foundation stock by only three years of artificial selection and maintained throughout in a common environment, should resemble so closely in several respects the geographic races of many mammalian species found in nature occupying opposite ends of a temperature cline (lowland vs. alpine; or Southern vs. Northern races in Europe and North America).

The positive association disclosed between body size and litter size is of such wide-spread occurrence in mammals that it is possible to generalize with the broad rule (MacArthur) that, within a species of mammals, litter size tends (with some exceptions) to average greatest in larger races of laboratory animals, in larger breeds of domestic animals, and in the larger geographic races of wide-ranging wild mammals (or birds) in nature.

We have given reasons for thinking that the same rule holds also for man, since the largest-statured human races certainly tend to produce large litters (that is, fraternal twins, triplets, etc.) most frequently. The incidence of fraternal twin births is three or four times higher among North Europeans, or West African (American) Negroes than among small races like the Japanese.

From the practical agricultural point of view, the qualities most prized in high-producing live stock are interrelated. Thus large body size, faster growth, more economical gains from feed and rapid and efficient reproduction are all intimately associated, and selection becomes an especially powerful tool for the breeder seek-

ing a simultaneous improvement in these useful characteristics.

SUMMARY

Small and large races of mice produced from one stock by eight generations of selection for body size alone, came to differ strikingly and significantly, not only in body size, but unexpectedly in many other characters and traits as well (behavior, hair colors, relative length of the appendages and litter size). The large race, for instance, has certain distinguishing coat colors (brown, dilute, etc.); is more docile and inactive; has comparatively shorter ears, feet and tail; and bears many more young in a litter.

The coat colors are such as are controlled by genes, either known to exert pleiotropic growth-accelerating effects, or probably fixed by chance drift. Behavior differences may be associated with metabolic levels. Appendages are proportionately small because they grow less rapidly than the body in the last stages of growth. Large litters are due to superovulation in the large race, evidently regulated by the gonadotropic hormone of the anterior pituitary.

Litter size and length of appendages appear to be dependent, like satellites, upon body size as the central and dominating member of a complex of correlated characters, with a common developmental basis in growth. Differences between the mouse races in litter size or length of appendages are determined, not by special fertility factors or ear-length genes, etc., but, in great part at least, by the same common and general multiple size or growth rate factors that control body size.

Several applications to general biological problems are suggested.

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THE CORRELATION BETWEEN ANTIGENIC COMPOSITION AND GEOGRAPHIC RANGE IN THE OLD OR NEW WORLD OF SOME SPECIES OF COLUMBA[1]

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INTRODUCTION

VARIABILITY of biological forms associated with geographical location has come to be a subject of primary and increasing importance in biology. Studies on this subject have been made largely on races or subspecies of an organism, although there are many which are concerned with diversity of species according to habitats. Several excellent reviews have recently been written on this subject and to these the reader is referred for discussions of the various findings and for references to the literature. Among such reviews are those by Dobzhansky (1941), Huxley (1942), Mayr (1942) and Timofeeff-Ressovsky (1940).

During investigations of the interrelationships of the cellular antigens of 11 species of the genus Columba, it was noted that there was a tendency for the species to fall into one or the other of two groups. Furthermore, the native habitat of the members of one of these two groups was in the Eastern and of the other in the Western Hemisphere. The analysis of the data on the association of these antigenic characters with habitat in the two hemispheres is given in this paper. Naturally, any statements which are made in this paper are applicable only to the species which have been tested.

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[3] The authors are indebted to Professors L. J. Cole and R. A. Brink for reading this manuscript and for their comments on it.

Various reports from this laboratory have shown that the cellular antigens, which distinguish the blood cells of one species from those of another, segregate in the back-cross offspring as would be expected if these characters are genetically determined (Irwin and Cole 1936, 1940; Irwin, *et al.* 1936; Irwin 1939). These and all studies by other workers on cellular antigens in other species make reasonable the conclusion that all the antigens of the blood cells in the various species tested—and probably those in all species—are gene-determined.

If this conclusion be true, it follows that the interrelationships of the cellular antigens of related species would be in effect an analysis of these various species in terms of the genes which have biochemical effects. The relationships of the cellular antigens of human cells to those of the higher apes and monkeys are discussed by Wiener (1943). The geographical distribution of the antigens (commonly called "blood groups") $\overline{A}$, $\overline{B}$, $\overline{AB}$ and $\overline{O}$ of humans is also discussed by this author. For a more detailed account of this latter subject, see Boyd (1939).

A recent paper (Irwin and Cumley, 1943) has analyzed most of the gross relationships of the cellular components of eleven species of Columba. This analysis has shown, among other things, that each of these species appears to be a biochemical entity in comparison with any other species, but has interlocking relationships of antigenic characters, and therefore of their causative genes, among all the other species. It is now proposed to examine the data of the above report for a possible correlation of the antigens of the species with their geographical locations. In so far as is known, the antigenic characters of the blood cells are not influenced by the environment and therefore represent more or less constant genic effects wherever they appear.

The details of the technique used in obtaining the agglutination reactions are described in the preceding paper (*loc. cit.*). Briefly, an antiserum to a particular species of Columba was absorbed by the cells of another species

and the resulting "reagent" was tested with the cells of all available species of the genus. This procedure was carried out with the antiserum to each of the different species, using in respective absorptions the cells of the other species.

The interpretation of the agglutination tests with the various reagents may be explained by an example. The antiserum to *flavirostris* cells was absorbed by those of *guinea*. This reagent agglutinated the cells of seven of the nine species with which it was tested, and from these results it can be stated that the cells of the species giving agglutination with this reagent did so by virtue of one or more antigens which each shared with *flavirostris* to the exclusion of *guinea*. And since this reagent did not agglutinate the absorbing cells (*guinea*) nor those of *palumbus*, one can deduce that *guinea* shares with *flavirostris* most if not all the antigens that this latter species shares also with *palumbus*.

Presumably, the closer the biochemical relationship between any two species, the greater will be the proportion of antigens which are *common* to them; and the proportion of these characters *specific* to either species will be correspondingly less. As the relationship between species decreases the relative proportion of *common* antigens also will decrease, and that of *species-specific* antigens will increase. The phrase "genes with effects on antigens of the blood cells" may be substituted for "antigens" in the preceding sentences.

It may be assumed that the present geographic distribution of the species of pigeons is correlated, in some measure, with the phylogenetic relationships of the species. With this in mind, the various species were arranged in terms of their geographic distributions in the hemispheres, as given by Peters (1937). The areas occupied by these several species are given in Fig. 1. The species of the New World used in these tests are *Columba fasciata, flavirostris, leucocephala, maculosa, picazuro* and *rufina*. Those of the Old World are *Columba guinea,*

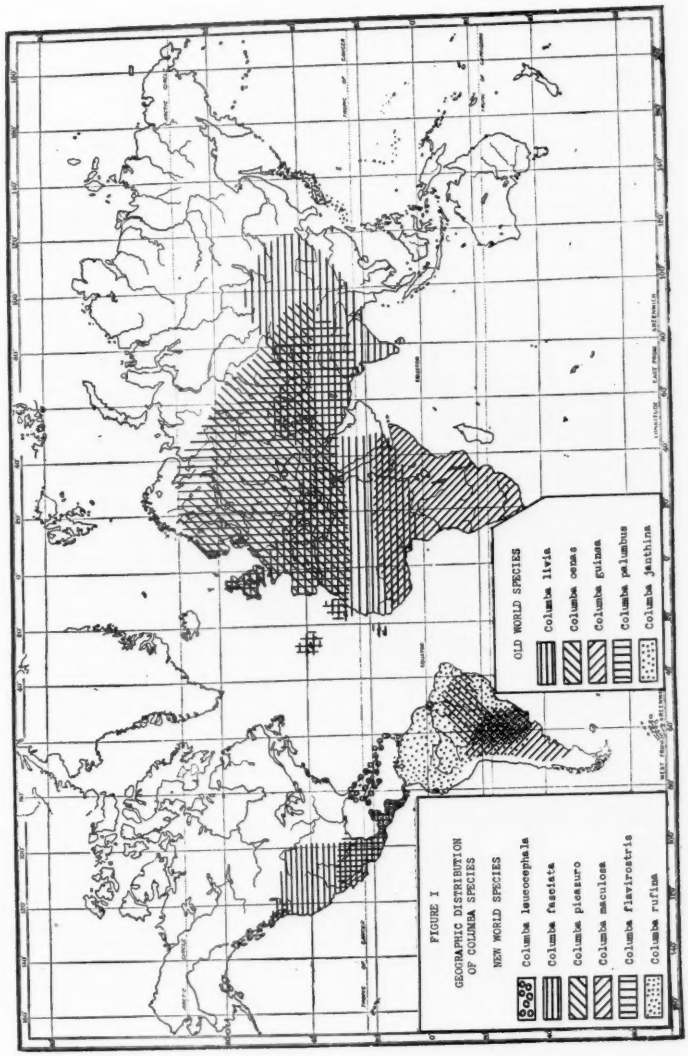
FIGURE I
GEOGRAPHIC DISTRIBUTION
OF COLUMBA SPECIES
NEW WORLD SPECIES
Columba leucocephala
Columba fasciata
Columba picazuro
Columba maculosa
Columba flavirostris
Columba rufina
OLD WORLD SPECIES
Columba livia
Columba oenas
Columba guinea
Columba palumbus
Columba jouthina

Fig. 1.

janthina, livia domestica, oenas and *palumbus.* None of the species native to the Old World has been found wild in the New World, and vice versa. (As is well known, the domestic pigeon of this continent, *C. livia domestica,* is an imported descendant of the *C. livia* which is native to Europe, Asia and northern Africa.)

TABLE I

A SUMMARY OF THE AGGLUTINATION REACTIONS OF REAGENTS, PREPARED FROM ANTISERUMS TO SPECIES OF COLUMBA, WITH THE CELLS OF ALL THESE SPECIES, GROUPED ACCORDING TO HABITAT IN THE OLD OR NEW WORLD, RESPECTIVELY

		A. A composite of the reactions of antiserums to species of the Old World after absorption by the cells of :												
		Old World species						New World species						
Cells used in tests	Cellular reactions	guinea	janthina	livia	oenas	palumbus	Totals	fasciata	flavirostris	leucocephala	maculosa	picazuro	rufina	Totals
Old World species	+	10	8	16	9	6	49	21	21	10	19	21	21	113
	0	1	0	0	1	1	3	0	0	0	0	0	0	0
New World species	+	15	12	24	16	7	74	15	22	0	19	8	5	67
	0	2	0	0	2	10	14	3	0	8	0	3	13	27

		B. A composite of the reactions of antiserums to species of the New World after absorption by the cells of :										
		Old World species					New World species					
		guinea	livia	oenas	palumbus	Totals	fasciata	flavirostris	maculosa	picazuro	rufina	Totals
Old World species	+	14	24	9	4	51	13	20	20	11	11	75
	0	3	0	0	0	3	4	0	0	1	6	11
New World species	+	29	36	30	14	109	20	20	20	13	8	81
	0	0	0	0	0	0	0	0	0	0	12	12

The digits represent the number of species of either the Old or New World, listed according to the reaction of their cells, either positive ("+") or negative ("0"), with the various reagents. See text for more complete explanation.

It will be noted in Fig. 1 that there is considerable overlapping in both continents of the areas inhabited by certain of the species. In the Eastern Hemisphere, for example, the ranges of *livia, oenas* and *palumbus* coincide in parts of Asia, southern Europe and northern Africa, and of *livia* and *guinea* in central Africa. So also do the

ranges of *maculosa, picazuro* and *rufina* overlap in South America, and of *fasciata, flavirostris* and *rufina* in Central America.

The data given in the table of the previous article (Irwin and Cumley, 1943) were summarized in two main groups, depending upon whether the antiserums were developed against Columba species of the Old or New World. Thus, in part A of Table I in the present paper, the various antiserums to the Old World species are grouped together, and the reactions following the absorptions by the cells of the respective species are listed, giving the number of both positive and negative reactions with cells of those inhabiting the Old and New World, respectively. Similarly, in part B of the table there is given the same kind of composite reactions of antiserums to New World pigeons with cells of species of both the Old and the New World. It is admitted that this division of reactions into the two classes (according to presence of absence of agglutination) does not give a complete picture of antigenic relationships, but it will serve as a starting point in the analysis of possible differences in the species according to habitat. Furthermore, it is conceivable that reagents prepared from other antiserums than the several to each species which were used in these tests might not always give the same reactions, or lack of them, as are summarized in the table. However, it is unlikely that the general picture of interrelationships among these species would be materially changed even if many more antiserums were employed in parallel tests. This statement can be made with reasonable assurance, for the application of the principle of reciprocal agreement of reactions, as explained in the previous paper, showed that the discrepancies were relatively few.

Old World Antiserums

The reaction of all antiserums, with the cells of the species of the Old and New World, against the several species of the Old World (*guinea, janthina, livia, oenas* and

palumbus) are combined in part A of Table I according to whether the absorptions were made by the cells of *guinea, janthina* or of one of the three other species. The results of the absorption of an antiserum by the homologous cells, as of anti-*guinea* serum by *guinea* cells, are not included in the summary. Nor are the reactions of an antiserum to the absorbing cells given as these, being usually negative, would tend to confuse the relationships. All questionable reactions in the original table are eliminated from this summary, as are those which were discrepancies according to the criteria explained in that paper. Thus, in part A of Table I, the antiserums against species of the Old World (*janthina, livia, oenas* and *palumbus*), when absorbed by the cells of *guinea,* gave agglutination of cells of Old World species in 10 combinations and no reaction in one. With cells of New World species, there were 15 positive reactions and two negative. If each antiserum to Old World species had been absorbed by the cells of all the other Old World species, there would be 16 combinations possible with cells of species of the Old World, other than the absorbing cells, and 24 with those of the New World. It will be noted in the table that the total number of possible reactions was but seldom observed, either because of discrepant reactions, involving only a small fraction of the total, or the lack of cells of a species at the time of testing. This latter condition was particularly true for the cells of *janthina, palumbus* and *leucocephala.*

The antiserums against the Old World species, when absorbed with cells of species of the Old World, were tested in a total of 52 combinations with cells of these species, resulting in 49 positive and three negative reactions. These same reagents were reactive in 74 combinations of cells of New World species and produced no agglutination in 14 such combinations. It is interesting that the absorptions by the cells of *palumbus* were responsible for 10 of these 14 negative reactions. That is, the cells of *palumbus* possessed many of the cellular antigens that

were shared by other species of the Old World with certain New World species. The differences in the number of positive and negative reactions of these reagents with cells of the Old and New World species, respectively, suggest that there is a definite tendency for at least certain of the cells of Old World pigeons to share all the antigens among themselves that are shared with these species of the New World. Indeed, the species of the Old World probably share *more* antigens among themselves than are shared with pigeons of the New World.

Much greater differences in reactivity of cells of the Old and New World species occurred following absorptions, by the cells of New World species, of antiserums to Old World pigeons. Agglutination was observed in each of 113 combinations of such reagents with cells of birds of the Old World as compared to 67 positive reactions and 27 negative reactions in 94 combinations with cells of New World species. These differences are highly significant. One of the most pertinent findings of this analysis of the data is that no species of the New World removed all the antibodies for cells of Old World species from antiserums against the Old World species. That is, none of these New World pigeons shared as many antigens with any species of the Old World as were shared *among* the species of the Old World.

On the other hand, each New World species except *flavirostris* and *maculosa* removed antibodies, for cells of other species than itself among those of the New World, from certain antiserums to species of the Old World. For example, there were 13 negative reactions among cells of the New World following absorptions by *rufina* cells of these antiserums to Old World species. Referring to the table with the complete data in the previous paper (*loc. cit.*), it may be seen that two New World species, *fasciata* and *rufina,* either removed antibodies for the cells of other New World species entirely from the antiserums to the Old World pigeons, or left antibodies capable of reacting with them only slightly, if one may

trust the degree of reaction to be a reasonable criterion. Unfortunately, only two of these antiserums (anti-*guinea* and anti-*livia*) were absorbed with *leucocephala* cells, but the reactions of these two reagents, combined with those following absorptions by the corpuscles of *fasciata* and *rufina,* suggest strongly that *fasciata, leucocephala* and *rufina* are very similar in content of cellular antigens shared with Old World pigeons. The cells of *picazuro* appear to contain a smaller quantity of these antigens, and those of *flavirostris* and *maculosa* a still smaller amount.

If the *common* substances represent more or less stable genic effects in the evolution of each of these species, it might be argued that *fasciata, rufina* and probably *leucocephala* have changed from the ancestral stock slightly less in these genes than did *picazuro,* and much less than did *flavirostris* and *maculosa.* Naturally, if these several New World species evolved from other than a common stock, more divergence would be expected than if but one ancestral origin had obtained. But the evidence strongly indicates that, although *flavirostris* and *maculosa* have different parts of the antigens of Old World pigeons which are *shared* by these six of the New World, the other species (*fasciata, leucocephala, picazuro* and *rufina*) possess all or nearly all of the combination of such antigens of these two species, plus others in addition. From this point of view the evidence points definitely toward a common ancestry of these six species of the New World.

However, in addition to the varying amounts of antigens shared with the Old World species and among themselves, each of these species has antigens which distinguish it from each of the other species tested. It is the variation in the amounts of cellular antigens shared with other species and the possession of specific antigens which distinguish it from any other single species that make each species a biochemical entity. Naturally, other biochemical characters, such as those of the serum, also will contribute to this end.

New World

A parallel summary of the reactions to antiserums of Columba species of the New World is given in part B of the table. Representatives of *janthina* and *leucocephala* were but seldom available for their cells to be used in the absorptions of these antiserums, although absorptions of *leucocephala* antiserum were made and the reactions to the reagents so produced are included.

It may be seen from these results that, following absorption of antiserums to New World species by the cells of four of the five Old World species, there were 51 positive reactions with cells of pigeons of the Old World and three combinations with no agglutination. The lack of reactions in these three combinations is a partial reflection of the close relationship of the cellular antigens between *guinea* and *palumbus,* as stated in the preceding paper (*loc. cit.*), since the cells of *guinea* by absorption removed antibodies from two antiserums for those of *palumbus.* Tests of these reagents on the cells of New World pigeons gave positive reactions in each of 109 combinations. That is, no pigeon of the Old World shared either the same or more antigens with any species of the New World than were shared with another of the New World. These particular results are in entire agreement with those of the reciprocal tests; i.e., reactions of antiserums to Old World species with the cells of these species, following absorptions by cells of New World pigeons.

But when cells of New World pigeons were used in absorption of the antiserums to species of this group, there were 75 positive and 11 negative reactions with cells of birds of the Old World, and 81 positive and 12 negative reactions with those of species of the New World. All the negative reactions of the latter group were obtained with reagents produced by absorptions with the cells of *rufina,* whereas either *fasciata* or *rufina* cells, and in one instance those of *picazuro,* removed antibodies for corpuscles of Old World species.

GENERAL CONSIDERATIONS

On the basis of the presence or absence of agglutinations of the cells of the various species of the Old and New World, it is clear that absorption of antiserums to pigeons of the Old World by cells of those of the New World did not completely remove the antibodies for any Old World species. A complementary situation was found in summarizing the reactivities of antiserums to New World pigeons in that absorptions by the cells of birds of the Old World did not remove all the antibodies for any of these species of the New World. It appears reasonable to conclude from these results that there are some antigens in these species of the Old and New World, respectively, that are not shared by any single species of the other hemisphere. (The possibility is not excluded, however, that such antigens might not be shared by a combination of two or more species from the other hemisphere. Data which test this possibility will be presented in another paper.) These relationships may be summarized as follows:

1. There are cellular antigens shared more or less uniformly by both Old and New World species of Columba.
2. Within the species of the Old and New World, respectively, there appear to be some antigens which are shared primarily within each group, but in varying proportions.

Thus, it appears that there are classes of antigens which are *common* either to all these species of Columba (category 1), or within the species of the Old or New World (category 2) in varying interlocking arrangements. In addition, each species definitely possesses antigens which distinguish it from every other single species. These might be described as being (3) antigens of species of either the Old or New World, portions of which are contained in many and perhaps in all species of the respective groups. A diagrammatic representation of these categories has already been given (Irwin and Cumley, 1940).

Some additional evidence on the relationship proposed above is provided if to the criterion of the presence or absence of agglutination is added that of the degree of reaction, or probable quantity of agglutination. Comparisons based on the degree of reaction are not as accurate indices of relationships as the presence or absence of a reaction, but should not be ignored in this analysis, particularly the questionable reactions (labeled "?" in the table in the preceding paper). The most plausible explanation of the questionable reactions is that the relationships, if any, dependent upon such reactions are likely to be slight. If these be added to the totals given in Table I, the results are as follows: For Old World antiserums absorbed by cells of species of the Old World, there were 6 questionable reactions of these reagents in tests with Old World cells, 6 with New World cells; if absorbed by cells of New World pigeons, there were no questionable reactions in tests with Old World cells, but 11 with New World cells. In the composite of antiserums to New World pigeons, following absorptions with cells of Old World species, there were 5 doubtful reactions in combinations with Old World cells, and none with those of the New World. If exhaustions were made by cells of New World species, there were 3 such reactions with Old World cells and one with those of the New World. None of these questionable reactions detracts from the argument of the division of these species into two groups according to habitat. Whatever deductions may be made from them would conform to those already proposed.

What appear to be the most obvious inconsistencies in the original table are the reactions of the reagents of anti-*livia* serum absorbed, respectively, by the cells of *janthina* and *leucocephala*. The agglutinations of Old World cells with this *leucocephala* reagent are of less degree than they are with the other, and thereby do not entirely conform to the postulate of group differences between Old and New World species. Whether this same kind of exception would have been found with other Old

World antisera cannot be predicted. The only other antiserum which was absorbed by the cells of these two species was that to *guinea*, and these reagents did not produce exceptional results in tests with the various cells.

Within these five species of the Old World group *guinea* and *palumbus* are the two whose antigens are most closely related, according to reasons given in detail in the previous article (*loc. cit.*). The other three species (*janthina, livia* and *oenas*) appear to be related to each other and to *guinea* and *palumbus* in varying degrees, depending upon the species with which the comparison is made. For example, the cells of *oenas* removed most of the antibodies from anti-*fasciata* and anti-*flavirostris* serums for the cells of both *guinea* and *livia*, showing that *oenas* shared with these two species most of the cellular antigens shared by them with *guinea* and *livia*. Leaving out the details of these complex relationships, it seems that these species of the Old World tend to resemble each other somewhat more than any one of them tends to resemble a single species of the New World. On the other hand, particularly in the antigenic complex which each species of the New World shares in varying amounts with those of the Old World, there is, as previously stated, evidence which very definitely suggests a common ancestry of these six species of the New World.

The results of the tests of antigenic relationships among these 11 species suggest strongly that, from the point of view of cellular antigens, these species of the Old and New World fall into two groups according to habitat in one or the other of the hemispheres. (A comparable grouping was proposed by Cumley and Cole (1942), using as a criterion the various color patterns, and including many more species than have been tested for antigenic relationships.) The species within each hemisphere have been shown by these tests to be definite biological entities, but still they are more alike *within* than *between* the groups. Within the species of the New World, *picazuro* and *rufina* are very closely related biochemically, as are *guinea* and *palumbus* in those of the Old World.

For reasons given in the previous paper (Irwin and Cumley, 1943), it is probable that the erythrocytes of *guinea* differ from those of *palumbus* by the products of one or more genes on each of three or four chromosomes. Although the actual number of chromosomes in these species of Columba is not known at present, the best estimate is that each has about the same number as was observed by Painter and Cole (1943) in *livia* and ring-dove (*Streptopelia risoria*), *viz.*, about 30 pairs. Genes on chromosomes of *guinea,* with antigenic effects, other than those on the three or four which distinguish *guinea* from *palumbus,* will then produce antigens *common* to these two species. These probably represent the effects of one or more genes on many, if not on all, of the chromosomes other than these three or four. At the moment there exist no criteria for estimating the number of cellular characters by which *palumbus* differs from *guinea,* or by which *picazuro* and *rufina* differ one from the other. It seems reasonable, however, to assume by analogy with the small number that distinguish *guinea* from *livia,* that there are relatively few involved in these other cases.

If the basic premise be admitted that these species of Columba have evolved from a common stock—and any other premise would be difficult indeed to maintain in the light of the data on the biochemical characters—one can postulate certain events that almost certainly have taken place in the evolution from the ancestral stock to the present species. For reasons already given in this paper, it appears justifiable to state that the species of the New World have evolved from a common ancestral stock. To account for the two main lines of divergence into species of the Old and New World, respectively, it may be assumed that there was a separation of forms within the common ancestral stock, and from one of these arose the species of the New World. But whether only two forms, or potential forms, existed at the time of this separation can be only a guess.

One can account most simply for the fact that in two closely related species, as *guinea* and *palumbus,* the ma-

jority of the genes which effect cellular characters now produce antigens which make the species alike, by alternate proposals or by a combination of these. Thus, disregarding the possibilities of position effect, either (a) the genes for cellular characters in these two species have changed very little in their evolution from the ancestral stock, or (b) whatever changes took place were for the most part parallel. To use a concrete illustration, we can cite the experimental findings which show that *guinea* differs biochemically from *livia* in the effects of one or more genes on each of five or six chromosomes (Irwin *et al.*, 1936), giving rise to antigens $\overline{A}$, $\overline{B}$, $\overline{CD}$, $\overline{E}$ and $\overline{F}$. Also, from unpublished data it appears that *palumbus* shares with *guinea* all of two ($\overline{A}$ and $\overline{F}$), parts of at least two more of these antigens ($\overline{CD}$ and E and possibly $\overline{B}$) which distinguish *guinea* from *livia*, as well as all or practically all the cellular components which are shared by *guinea* and *livia*. (It is possible to make the latter statement, since *palumbus* cells removed all or nearly all the antibodies from *guinea* antiserum for the erythrocytes of *livia*, as shown in line 26 of the table in the previous paper.)

If, then, *guinea*, *livia* and *palumbus* trace back to a common ancestral stock, it follows that, during their separation into distinct species, gene changes occurred in five or six chromosomes of either *guinea* or *livia*. Whether these changes from the ancestral form occurred primarily in *guinea*, or only in *livia*, or some in both cannot be answered definitely. As the changes from the ancestral stock occurred in *guinea*, in *livia*, or in both, to give the demonstrable genic effects ($\overline{A}$, $\overline{B}$, $\overline{CD}$, $\overline{E}$ and $\overline{F}$) which now set *guinea* apart from *livia*, *guinea* and *palumbus* pursued a parallel course in respect to certain antigens specific to *guinea* in relation to *livia; viz.*, $\overline{A}$, $\overline{F}$ and parts of the $\overline{CD}$ complex and $\overline{E}$, and perhaps of $\overline{B}$. Both species, however, underwent sufficient change in genic material to make each a biological entity with cellular characters peculiar to itself.

The interactions given in the table of the preceding article (*loc. cit.*) show further that *guinea* shares with *oenas* some antigens not found in *livia* (lines 23 and 67 of the table in the preceding article). Likewise, *guinea* and *livia* have in common cellular components not present in *oenas* (lines 25 and 53), and *guinea* shares with *palumbus* all or practically all the antigens which it shares with both *oenas* and *livia* (line 26). Furthermore, *guinea* has some cellular characters in common with *janthina* to the exclusion of either *livia* or *oenas* (lines 23 and 25), but shares with *palumbus* all or nearly all the substances which it (*guinea*) possesses jointly with *janthina* or *livia,* and possibly with *oenas* (line 26). From these relationships of cellular antigens the deduction seems justifiable that *guinea* pursued one evolutionary path in comparison with *livia,* a slightly different one with *oenas,* another with *janthina,* but that *palumbus* accompanied *guinea* all or almost all the way on each of these three routes.

In relation to the species of the New World, *guinea* shares substances with each of the six of this group to the exclusion of either *janthina* or *livia* (see lines 21 and 23 in the table of the preceding article), and shares with *fasciata, leucocephala, maculosa, rufina* and perhaps with *picazuro* but not with *flavirostris* some substances *not* held in common with *oenas* (line 25 of the table). Furthermore, as stated earlier in this paper, *fasciata, leucocephala, rufina* and probably *picazuro* contain the same, or most of the same, antigens that are shared with Old World pigeons in general and with *guinea* in particular, while *flavirostris* and *maculosa* have a lesser quantity of the common substances than do the other four species. To return to a consideration of the various evolutionary paths which *guinea* travelled in relation to the several species of the New World, one may state that *guinea* took only slightly different roads in relation to *flavirostris* and *maculosa,* respectively, and that the other four species of the New World—*fasciata, leucocephala, picazuro* and *rufina*—accompanied *guinea* on these routes and to a more

or less common point beyond. Again referring to the known genetic characters ($\overline{A}$, $\overline{B}$, $\overline{CD}$, $\overline{E}$ and $\overline{F}$) by which *guinea* differs from *livia,* it has been found that all or parts of $\overline{A}$, $\overline{CD}$ and $\overline{F}$ are shared by *guinea* with most of the New World species. The occurrence of these or closely related cellular substances ($\overline{A}$, $\overline{CD}$ and $\overline{F}$) in various species of both Old and New World makes it appear that these particular genetic characters are those which have persisted throughout their evolutionary course in these several species whereas the changes from these have taken place in *livia.* Such genic effects along with those *common* to *guinea,* or other species of the Old World, and the New World pigeons would probably be those of greatest age in the several species. Otherwise one would need to hypothesize parallel mutations to explain their occurrence in these various species. It seems highly probable that the genes which produce substances *common* to any two species are more numerous than those producing the antigens *specific* to either species, so the possibility of parallel mutations accounting for the block of cellular components *common* to both Old and New World species seems rather remote.

The principal steps in the evolutionary paths of each of the other species of the Old World could be traced in the same manner as described for *guinea,* with the expectation that for each species a particular pattern with respect to the others would be found. And since comparable interrelationships exist within the various species of pigeons of the New World, from them may be deduced an outline of the evolutionary pattern for each species of this group. However, such deductions will be more exact if further tests are made of these antigenic relationships, particularly the extent to which a species may share antigens with another to the exclusion of two others, as will be shown elsewhere. (It is particularly unfortunate that the cells of *janthina* were not always available for testing. Occupying as it does a more or less isolated habitat off the eastern coast of Asia, the interrelationships possible with

the other species might have shown whether this species represents a bridge between Old and New World forms, as might be suspected if the pigeon species spread from the Old World to the New via eastern Asia.)

What appears to have taken place in the evolution of these species in the different hemispheres is probably a large scale model of how the evolution of other species may proceed within a smaller geographic range. But for these species of Columba there does not appear to be a correlation between closeness of antigenic relationship and present habitat *within* the hemispheres. As stated above, the species of the Old World which are most closely related antigenically are *guinea* and *palumbus*. Their ranges do not overlap, according to present information, yet each coincides in range with at least one other species of a less degree of this kind of biochemical relationship. Similarly, in the New World, although *picazuro* and *rufina* are the two most closely related, *rufina* shares geographic range with *maculosa* almost to the same extent as with *picazuro*. Nearness of habitat, then, need not necessarily be an index of degree of relationship of species, at least not of biochemical relationship.

Summary

An analysis of the data on antigenic interrelationships of the blood cells among eleven species of the genus Columba indicates that these species tend to fall into two groups. The species comprising one of these groups lives in the Old World, those of the other in the New. The reasons for this grouping are based primarily upon the data which are interpreted as indicating that the antigens of each species of either the Old or New World are more like those of any other species of that hemisphere than they are like those of any single species of the other. All available evidence points to the conclusion that the cellular antigens of a species are many, and are gene-determined. On this basis, it appears that a separation took place in the ancestral stock early in the evolutionary his-

tory, and that the species of the New World evolved from one of the forms, those of the Old World from another.

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THE DISTRIBUTION OF THE OSMIINE BEES OF THE DESERTS OF NORTH AMERICA

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IN the course of revisional studies of various genera of Osmiinae a repetition of certain distributional patterns was observed in all genera characteristic of the North American desert regions. It is the purpose of this paper to present the data involved, which is derived from a study of nearly four thousand specimens from the arid regions of the southwestern United States, and to suggest possible reasons for the peculiarities of distribution noted.

The Osmiinae are solitary bees which nest in the soil, in wood or, more rarely, in snail shells, unused portions of mud wasp nests, etc. As with all bees, they are entirely dependent upon flowers for food. Many of the species of the subfamily are confined in their pollen collecting to a few, usually related, species of flowers and are called oligolectic. Such species usually have but a single brood each year, the winter being passed in the prepupal state and emergence of the adults taking place at the season when certain favorable flowers are in bloom. After a few weeks of activity, new nests are provisioned, eggs laid in them, and the adults die. Species with longer seasons of flight, often with two or even more broods each year in some areas, commonly collect pollen from many unrelated species of plants. These bees are termed polylectic.

The bees of the desert areas may be arbitrarily divided into two overlapping groups based upon their season of flight and the nature of the plants upon which they feed. The first of these groups consists of genera which, in the desert, fly primarily in the spring and are dependent upon the flowers of herbaceous mesophytes which grow up and bloom during the short period when the desert is relatively moist. The bees of this group are primarily Hol-

tory, and that the species of the New World evolved from one of the forms, those of the Old World from another.

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The bees of the desert areas may be arbitrarily divided into two overlapping groups based upon their season of flight and the nature of the plants upon which they feed. The first of these groups consists of genera which, in the desert, fly primarily in the spring and are dependent upon the flowers of herbaceous mesophytes which grow up and bloom during the short period when the desert is relatively moist. The bees of this group are primarily Hol-

arctic types, such as *Andrena, Dufourea* and *Tetralonia.* The second group consists of genera which visit the flowers of the characteristic xerophytes, such as *Larrea, Prosopis* and *Cercidium,* not only in the spring but also later in the year. This second group includes forms whose nearest relatives are Neotropical (such as *Centris, Ericrocis, Protoxaea* and apparently *Perdita*), as well as representatives of such widespread genera as *Halictus* and *Megachile.* There is scarcely a genus of bees every species of which may be unquestionably placed in one or the other of these groups; nevertheless, the division is quite evident. The Osmiinae adapted to the deserts fall for the most part in the second group, although they, like the characteristic elements of the first group, are derivatives of Holarctic genera. This northern origin of the Osmiinae is evident from their distribution. They are found in the Holarctic and Ethiopian faunal regions; no species of the subfamily is known from South America or Australia.

It appears probable that the group of genera here considered (Tribe Osmiini[1] of Michener, 1941, *Am. Midl. Nat.*, 26: 147–167) arose in the Palearctic region, because it is there that the species of some of the widely distributed and morphologically apparently primitive genera are most numerous and diversified, and it is there also that the lines of separation among these genera become evanescent. Indeed some genera are so closely related that it seems certain that they must have arisen from ancestors which would fit our definition of extant genera. Thus it is probable that the characteristics which delimit *Hoplitis* were in existence in the ancestral stock at the time when *Anthocopa* was differentiated.

Since the characters used in determining the phylogenetic relationships of the genera and a phylogenetic tree

[1] It now appears probable that this group of genera is not best regarded as a tribe. However, it constitutes a monophyletic group. Genera of the subfamily Osmiinae outside of this group scarcely invade the edges of the desert areas.

for them have been presented in a previous paper (Michener, 1941), it seems unnecessary to repeat them here.

Osmia, Hoplitis and Diceratosmia

These are the genera of the Tribe Osmiini which are rare in the deserts, being represented there by relatively few individuals of a small number of species closely related to or identical with species found in adjacent more humid regions. A brief discussion of them is included merely to contrast these forms with those considered below. *Osmia, Hoplitis* and *Diceratosmia* are all Holarctic genera. Both *Osmia* and *Hoplitis* are abundant in species as well as in individuals in the mountains east and west of the desert areas and in the cool mesophytic areas north of them. Their rarity in the desert contrasts strongly with the greater abundance of many genera of aculeate Hymenoptera in the arid regions than elsewhere. *Diceratosmia* in North America is found only east of the Rocky Mountains except for a single species extending into the southwestern deserts.

Anthocopa

The American forms of the genus *Anthocopa* are confined to the area west of the eastern foot of the Rocky Mountains. The five American subgenera are probably derived from two ancestral forms of *Anthocopa* which migrated independently into northwestern America from Asia. The subgenera *Atoposmia* and *Hexosmia* are montane and northern groups, probably similar to these ancestral types. The first is confined in its pollen collecting to *Pentstemon*, the second to Hydrophylaceae. Each of these subgenera, or an ancestral form similar to each, has apparently given rise independently to distinctive desert derivatives. Thus *Eremosmia*, a desert subgenus closely related to *Atoposmia*, contains about six species which are oligolectic on various Hydrophylaceae and Leguminosae. *Phaeosmia*, confined primarily to the desert, is a subgenus of four known species, all of which

collect pollen from Compositae. The relationship of this subgenus to *Hexosmia* is comparable to that of *Eremosmia* to *Atoposmia*. *Xerosmia* is a very distinctive desert subgenus, probably derived from a *Hexosmia*-like ancestor. The two species of *Xerosmia* are oligolectic on species of *Cryptantha*, and as in the genus *Proteriades*,

FIG. 1. Map of North America, showing the distribution of the subgenera of *Ashmeadiella*. Line 1 encloses the area occupied by *Ashmeadiella* s. str.; 2, *Arogochila;* 3, *Chilosima;* 4, *Corythochila;* 5, *Cubitognatha*. The known localities for *Titusella* are marked by x. (The cut for this figure is used through the courtesy of the *American Midland Naturalist* and its editor, Dr. T. Just.)

which also feeds on this plant, the mouthparts are short and furnished with numerous hooked bristles. I do not believe that this similarity in mouthparts is indicative of a close relationship between *Proteriades* and *Xerosmia;*

rather it seems likely that the resemblance is a result of convergent development.

The three desert subgenera of *Anthocopa* thus far have not been collected outside of California; indeed, most of the specimens taken have come from locations in the desert within easy view of the mountains which mark the western edges of the desert areas. Thus, although one species of *Phaeosmia* occurs in the coastal part of southern California, all other species belonging to the subgenera characteristic of the desert occur in a narrow zone along the western margins of the Great Basin and Colorado Desert, scarcely larger than that indicated for *Cubitognatha* in Fig. 1.

It is an interesting sidelight that the genera *Megandrena* and *Ancylandrena*, whose relationship to the primarily Holarctic genus *Andrena* is nearly as close as that of *Eremosmia* and *Phaeosmia* to the other subgenera of *Anthocopa*, are restricted to the same small area occupied by *Eremosmia* and *Phaeosmia*.

Ashmeadiella

The genus *Ashmeadiella* is widely distributed in North America, ranging from southern Canada to Yucatan and from the Atlantic to the Pacific, but is well represented only in the western part of its range. The close relationship of the species of *Ashmeadiella* and the limited distribution of the genus as a whole is evidence that it is a younger genus than *Anthocopa*, from which it is apparently derived. The wide distribution and morphological diversity of *Anthocopa* suggest relative antiquity. *Ashmeadiella* is divisible into several subgenera. The subgenus *Ashmeadiella* s. str. appears to be the most primitive of the subgenera, being similar morphologically to certain groups of *Anthocopa*. The details of the subgeneric characters and a phylogenetic tree have been omitted in this paper since they were presented previously (Michener, 1939, *Am. Midl. Nat.*, 22: 1–84).

In connection with the theory that the subgenus *Ashmeadiella* s. str. is more primitive than the other sub-

genera, it is interesting to note that many of its species are polylectic and fly more or less continuously through much of the summer. Thus *A. californica* (Ashmead) has been collected by P. H. Timberlake at Riverside, California, from April 11 to November 1. On the other hand, the majority of the species in the other subgenera are oligolectic and have short seasons of flight, a condition characterizing some species of the subgenus *Ashmeadiella* s. str. as well.

When plotted on a map (Figure 1) the distributions of the various subgenera form a series of concentric areas whose common center is the western part of the Great Basin and western Colorado Desert, in which region species as well as individuals of all of the subgenera (except *Titusella*) are most numerous. While the rare and little-known montane subgenus *Titusella* is properly excluded from this paper on desert bees, its dispersal probably having been hindered rather than facilitated by the desert areas, it is included in the discussion for the sake of completeness. In the following list the subgenera are named in the order of decreasing area occupied with the number in parenthesis after each indicating the number of species and subspecies included: *Ashmeadiella* s. str. (39), *Arogochila* (15), *Titusella* (4), *Chilosima* (2), *Corythochila* (2) and *Cubitognatha* (1).

PROTERIADES

The genus *Proteriades*, unlike the previously mentioned genera, is closely related to the Holarctic genus *Hoplitis* and occurs only in California and, no doubt, certain adjacent states. It consists of probably fifteen or twenty species, all oligolectic on species of *Cryptantha* and having specialized mouthparts probably adapted for use in flowers of this plant.

Although a few species occur in the coastal parts of California, the majority are found in the western Great Basin region and in the Colorado Desert. It is in these desert regions, also, that the greatest morphological diversity is found.

Discussion

It will be seen from the foregoing that a least four groups of Osmiinae have independently adapted themselves to desert conditions in western North America. These groups are (1) *Anthocopa,* subgenus *Eremosmia;* (2) *Anthocopa,* subgenus *Phaeosmia* and probably *Xerosmia;* (3) *Ashmeadiella* and (4) *Proteriades.*

The distributional problems involved are twofold. First, why are the desert groups concentrated in or restricted to the extreme western edge of the desert? Second, why are the distributional areas of the subgenera of *Ashmeadiella* concentric with the western margin of the desert as the center?

Both questions might be answered by assuming that the desert forms are relicts of groups more widely distributed at a time when the desert areas were larger but limited now to a favorable area, some subgenera of *Ashmeadiella* being more restricted than others. Such an explanation is difficult to believe, since we are dealing with genera containing many closely related species and having every appearance of being relatively young. One would also expect some of the relict species to occur in the deserts of Arizona and New Mexico which in most elements of their biota are very similar to those of California; yet it is only the more widespread subgenera of *Ashmeadiella* which are found in those areas.

It is easier to believe that the desert groups originated in the region where now they are most abundant, that is, on the western edge of the desert, and spread thence in varying degrees over the arid regions and even into relatively humid areas. It is then necessary to determine why parallel desert groups should not have arisen elsewhere, for example in New Mexico where the Rocky Mountains meet the desert areas.

A possible explanation of the absence of desert groups of Osmiinae in other areas is that, guided by the north and south mountain ranges and climatic zonation, the so-called ancestral genera, after entering North America

from Asia, migrated rapidly southward along the Pacific coast far into California before they were able to spread eastward into the interior states. The Osmiine genus *Chelostoma,* found in America only in our Pacific states, is an example of a genus still exhibiting such a distribution. A genus so distributed would first come in contact with arid regions in the particular area where the desert groups apparently center. There, in a region of very steep topographic and climatic gradients, it would perhaps give rise to desert derivatives such as the groups under consideration. Eastward and subsequent southward spread of the ancestral genera along the Rocky Mountains might have taken place much later than the southward dispersal along the Pacific coast, so that contact with desert conditions in the southern Rocky Mountain region may have taken place so recently that no distinctive desert groups have developed there. Such a theory requires that the desert groups be not older than Miocene, since the desert itself is of that age. It may be that *Ashmeadiella* arose in this way considerably earlier than the others, so that certain species have had time to readapt themselves to moderately moist climates and have spread over much of North America.

While it seems probable because of the similarity in distribution that the various desert groups of Osmiinae arose in the same manner, it may, nevertheless, be true that one of the foregoing hypotheses would apply best to certain groups, while the other might explain the origin of others.

Assuming, then, that the desert genera and subgenera originated where they now occur, let us return to the distribution of *Ashmeadiella.* The facts presented above suggest that from a center of origin in the western Great Basin and western Colorado Desert, the various subgenera, with the probable exception of the montane *Titusella,* arose and radiated as a series of waves over the continent. The one which produced species having the greatest collective tolerance to environmental conditions (the most primitive and presumably the oldest) adapted

itself to habitats covering the greatest territory, whereas the most highly specialized, perhaps the youngest, is narrowly restricted. Yet each remains in the entire territory which it populated, not being replaced by succeeding waves. If this is a correct explanation, it is indeed remarkable. Why should not some of the subgenera appear at and radiate from other points? Perhaps *Titusella* is an example of a group which did arise at a point other than the center of origin of *Ashmeadiella*. A possible explanation for the apparent common center of origin of the various subgenera may involve the climatic extremes and intense solar radiation of the region, factors possibly responsible in part for extensive speciation there. It is, moreover, a region of many isolated mountain ranges with adjacent diverse habitats which would perhaps favor selection of biotypes and perhaps eventual geographic isolation of new forms. Furthermore, in the apparently favorable environment of the western parts of our desert areas there has been the largest number of individuals and hence the greater chance for mutations and possibly for the development of new types which might eventually become additional species and even subgenera.

Since the powers of dispersal of bees appear to be great, it seems very improbable that the restricted ranges of the various species and subgenera should be entirely due to lack of time necessary for further migration. It is likely that a species will rapidly occupy the entire area environmentally suited to it and not separated from it by impassable barriers. Changes in climate or topography or in the tolerance of the species for environmental factors would then be necessary in order further to enlarge the range. Therefore, factors other than the length of time required for dispersal are probably responsible for the limited distribution of certain species and subgenera. The extent of the dispersal probably depends more on the rate of production (or the time available for the production) of new biotypes adapted to new environmental conditions than on the time available for dispersal.

The migrations of the subgenera must have taken place since the last major climatic changes in the area concerned; otherwise the clear-cut distributional pattern of concentric areas would have been disturbed. To a large extent the region now occupied by *Ashmeadiella* was not greatly affected by Pleistocene glaciation, and the dispersal of *Ashmeadiella* could have continued with little interruption through this period. If the hypotheses presented are correct, they may lend some confirmation to parts of the much criticized "Age and Area" theory of Willis.[2] Possibly such distributional effects can be recognized only in those groups which have spread rapidly in areas in which climatic and topographic changes have been slight during the time of dispersal.

SUMMARY

With the exception of four groups, the American Osmiinae are northern and montane forms, rare in the deserts and virtually absent in the neotropics. These four groups, independently derived from northern forms, are characteristic of a zone in which they probably arose along the western margin of the desert of central and southern California. Three of the groups are largely confined to this zone. The fourth, *Ashmeadiella*, has a broader range, covering most of North America, but is divisible into several subgenera, the distribution of which form concentric areas (Fig. 1) whose common center is in the region referred to above. Individuals and species of the more widely distributed subgenera are most abundant in this area. The morphologically generalized subgenus is the widespread, largely polylectic one containing many species, while the more restricted subgenera consist of fewer species, which are for the most part oligolectic. This distributional pattern suggests the possibility that from a common center of origin in the western Great Basin and Colorado Desert, the subgenera arose and radiated.

[2] J. C. Willis, "Age and Area," x + 259 pp., Cambridge Univ. Press.

REVIEWS AND COMMENTS

EDITED BY CARL L. HUBBS

In this section reviews and notices are given of current publications on general biology and of specialized works which have an important bearing in this general field. Emphasis is given to books and major articles which fall within the special scope of The American Naturalist, in that they deal with the factors of organic evolution.

Reviews and Comments are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as otherwise indicated, all items are prepared by the Section Editor, Dr. Carl L. Hubbs, University of Michigan, Ann Arbor, Michigan. All opinions are those of the reviewer.

A Contribution to the Theory of the Living Organism. By W. E. Agar. Melbourne: Melbourne University Press (in association with Oxford University Press), 1943: 1–207. 12s, 6d.

Philosophy, psychology and biology are combined in this interpretation of life processes. The ideas, in so far as they diverge from ordinary concepts, rest largely on Whitehead's panpsychism. That philosophy, though so heavily relied upon, is admittedly difficult to understand—a vast understatement.

Perception and appropriate behavior are central themes in Agar's theory: organisms and their parts are "subjects," which perceive stimuli, anticipate consequences and react accordingly. Embryonic development is regarded as a complex of instinctive behaviors. Purpose, striving, feeling and satisfaction are ascribed to the activities of all but the simplest animals and of plants; and are also attributed to the physiological functions of individual cells and of higher "nexus" (tissues and organs). There is a hierarchy of "agents" intermediate between the cells and the "Central Agent," which is the individual animal, integrated chiefly by the nervous system. It is even suggested that what are ordinarily treated as attributes of the human mind are involved in the compounding of chemical substances, molecules and atoms. In the lower organisms and substances the brain is replaced by a "mental pole."

It is repeatedly pointed out, however, that these psy-

chological terms are used without any connotation that the organs, cells and lower organisms feel, perceive, anticipate and react with consciousness. Their "feelings," however, are the evolutionary antecedents of consciousness. The actual functions of all organisms are admittedly physical, but through evolution, purposive function to an increasing degree has become a characteristic of life processes. Life therefore transcends the simple materialism of Loeb.

The restricted connotations which are ascribed to the psychological terms lead the reader to wonder at times whether the differences between Agar's philosophy and current biological thought may not lie largely in the choice of words. He applies to the activities of lower organisms terms that are ordinarily associated with the human mind. Others describe the same acts in physical terms. Differences in interpretation are vastly less fundamental than the respective terminologies would indicate. Nevertheless, there is some real distinction in concepts, at least differences of degree, for Agar projects supposedly human attributes backward into the evolutionary series farther than most biologists would.

Agar applies a teleological view only to the direct attainment of "hormic goals," through specific responses and physiological functions. There can be no striving toward the satisfaction of other than immediate needs. It is claimed to be beyond the realm of science to ascribe purpose to life processes, including those of ontogeny and phylogeny, which lead indirectly and remotely to favorable biological consequences. Apparently it is beyond the mental powers of lower organisms and cells to look that far forward.

The theory of natural selection is held to provide the only plausible explanation for these processes leading to remote consequences. The Lamarckian idea on which other psychobiologists have rested their case is regarded as untenable, because even if acquired characters be accepted as heritable, the Lamarckian factor must be dras-

tically limited. Since on Agar's theory there can be no "striving" toward remote functions Lamarckian inheritance and evolution can not be attributed to the many biological processes which lead indirectly—as in the ontogeny of the eye—to favorable biological consequences.

Plants and Vitamins. By W. H. SCHOPFER. Authorized translation by N. L. NOECKER. Waltham, Mass.: The Chronica Botanica Co.; New York: G. E. Stechert and Co., 1943, i–xiv, 1–393, 3 pls. $4.75.

WITH the tremendous interest in the subject of vitamins exhibited by plant physiologists in recent years this book should be welcomed by them as well as by others interested in the subject, and no one is better equipped to write such a book than the author, who has been a pioneer in this field.

The author has divided his book into three sections. In section one, "Synthesis of Vitamins in Plants, Auxoautotrophic Plants, Research Methods," he takes up the synthesis and the uses of vitamins by the green plants. After discarding many proposed definitions for vitamins he proposes a definition of his own. A vitamin is "an organic substance, the need for which results from the loss of the capacity for its synthesis, whose action is catalytic (active in small amounts), quantitative, and markedly specific." Section two, "Vitamins in Relation to Plants Unable to Synthesize Them, Growth Factors of Microorganisms," takes up in considerable detail investigations on the nutrition of such organisms as yeasts, lactic acid bacteria, Staphylococcus, etc. Also the function of some vitamins as coenzymes is discussed. Some pages are devoted to vitamins in relation to growth hormones. Section three, "General Problems Involving Vitamins," deals with vitamins in the soil and fertilizers, external factors influencing the vitamin content of food plants, vitamins and sexuality and symbiosis. A chapter is devoted to microrganisms as test objects for vitamins, but the author does not give any details of procedure. In the opinion of the reviewer this should have been done.

The author has made an attempt to correlate and explain some of the plant growth phenomena familiar to the plant physiologist in terms of vitamins, and the reviewer believes he has succeeded to a considerable extent. Again and again the author points out the unsatisfactory state of our knowledge of vitamins and this is bound to act as a stimulus to further research on the part of the reader. A very important function of any book should be to stimulate and encourage further research for the truth, and in this respect Professor Schopfer's book should prove of much value. A criticism should be offered because of an insufficient bibliography. In too many instances he refers to the work of certain authors but no citations are made and the reader will be able to locate the papers referred to only after consulting other authors.

F. G. GUSTAFSON

Studies in the Genetics of Drosophila. III. The Drosophilidae of the Southwest. Directed by J. T. PATTERSON. Univ. Texas Publ., 4313, 1943: 1–327, col. pls. 1–10, pls. 1–15, figs. 1–66, maps 1–16. $2.50.

SYSTEMATICS, life history, morphology, zoogeography, population biology and cytology are all advanced in this contribution, which helps to break down the walls between these cubicals of zoology.

The major portion of the volume is devoted to a systematic analysis of the Drosophilidae of the Southwest, by J. T. PATTERSON. The field explorations were very extensive and the species were thoroughly studied. Many are illustrated on the ten handsome colored plates. Other illustrations portray the male and female reproductive systems, the egg and the puparium, all of which exhibit valuable characters. As a result of the studies by Patterson and others the systematics of the Drosophilidae now rivals that of the Culicidae. The relative abundance of the species in various Southern, Southwestern and Mexican states is tabulated. The total records of identified individuals run into many thousands. For one plot in Texas very extensive data were secured on the seasonal

fluctuations in the abundance of the species of *Drosophila*. Temperature is shown to be a factor of prime importance.

The geographical distribution of the species of *Drosophila* is treated by J. T. Patterson and R. P. Wagner. Locality records are spotted on 16 maps, and the ranges are stated. The distributions are analyzed by regions and to some extent by habitat. Widely distributed species are discussed, and the role of introductions is considered. The relative abundance of various species in different populations is tabulated at length. The relationships of the Nearctic forms are held to lie with both the Palearctic and the Neotropical types. Since the Neotropical Realm has been intensively explored for *Drosophila* only in Mexico, it is quite possible that what is here called "Neotropical" may refer to an independent, Middle American biota, rather than to one of South American origin. Middle American types are numerous and diverse in certain groups, such as the fresh-water fishes.

In the last paper of the series Linda T. Wharton presents a significant comparative analysis of the metaphase and salivary chromosome morphology in 88 strains of *Drosophila*. One general conclusion is that gross alteration of the metaphase chromosome structure is not always paralleled by an equivalent change in the salivary chromosome. Another point is that wide alterations in the arrangement of the chromosome elements have occurred during the divergence and evolution of the species of *Drosophila*.

Statistical Analysis in Biology. By K. Mather. New York: Interscience Publishers, Inc., 1943: 1–247, figs. 1–9. $4.50.

"It is very rarely that the full value of a biological experiment can be realized before the observations have been subjected to a suitable statistical analysis." On this well-warranted assumption the author presents anew the common methods of statistical analysis. As Snedecor did, he follows Fisher very closely. That he has properly

interpreted that master of modern statistics is assured in the Foreword, written by R. A. Fisher himself: "The present work, designed as a more general introduction to statistical methods for biological investigators, shows the same practical grasp of the essentials of good experimentation [as does Mather's previous book, 'The Measurement of Linkage in Heredity'], and the same deliberate avoidance of what is extraneous. It is very simply written. . . ." The last statement is interesting, as a quite different appraisal is often heard expressed toward Fisher's own books.

A second assumption, that "statistics is the mathematics of experiment," may be challenged. Statistics is a tool of research, quite as valuable in observational as in experimental science. It is the mathematics of racial analysis and of economics quite as much as that of experiment. Biologists following the observational methods—for example, human biologists and systematists—may find the treatise by Simpson and Roe more pertinent to their needs. Experimental biologists, however, will have in Mather's new book an excellent statement of current statistical methods.

Mather follows what have now become the established traditions of statistical writers: proposing a new set of symbols, and failing to give any list or index of them. He recommends that "in presenting the results of any test of significance the probability itself should be given," but follows the custom of placing special reliance on certain levels of probability ($P = .01$ and $P = .05$). Tables are now available by which one may obtain precise estimates of P, and it is high time that biologists fully realize that values of P between .05 and 1.00 are of significance, to a degree of reliability that is inversely proportional to the magnitude of the value. There are no levels at which the indication of significance suddenly changes from one of complete reliability to one of moderate acceptability and thence to one of no reliability at all. Evidence needs be weighed to more than two or three degrees.

NOTICES OF NEW BOOKS

An Outline of General Physiology. Second Edition, Revised. By L. V. HEILBRUNN. Philadelphia and London: W. B. Saunders Co., 1943: i–xii, 1–748, figs. 1–135. $6.00.—The prodigious amount of scholarly work which went into the first edition of this treatise has now been supplemented by a thorough digest of recent advances. New sections deal with "the electron microscope; localization of enzymes in cells; colloid chemistry of the nucleus; tracer elements, and carbon dioxide syntheses. The greatest changes are to be found in the completely rewritten chapters on enzymes, vitamins, and protoplasmic oxidation." With two thousand new references it is more than ever a synthesis of the science of general physiology. It is the product of a dynamic investigator, who presents his subjects as a living, growing entity. The author's own researches and theories on such fundamental subjects as protoplasmic viscosity, anesthesia, the colloid chemistry of protoplasm and the physiological roles of calcium are given some degree of preeminence—but would one expect or want it to be otherwise?

The Permeability of Natural Membranes. By HUGH DAVSON AND JAMES FREDERIC DANIELLI. Cambridge: at the University Press; New York: The Macmillan Co., 1943: i–x, 1–361, figs. 1–73. $4.75.—"Organisms could not have evolved without relatively impermeable membranes to surround the cell constituents. This barrier between the inside and the outside, the inner and external world of each living unit, has been and always must be considered one of the fundamental structures of a cell. . . . There can be no doubt of the fundamental importance of cell permeability . . . but no general books on cell permeability have appeared for over ten years. . . . Cell permeability had passed from the qualitative to the quantitative stage and the detailed data now available would baulk less enthusiastic authors, even in normal times. . . . Cell physiology will be grateful indeed for this summing up of a subject which is destined for rapid development under the stimulus of modern methods of exploring molecular dimensions and molecular arrangement. Viewpoints may differ but the facts remain. These are systematically and logically presented in this timely volume."—From the Foreword by E. NEWTON HARVEY.

A Guide to Bird Watching. By JOSEPH J. HICKEY. Oxford University Press, New York, 1943: i–xiv, 1–262, drawings by F. L. Jaques. $3.50.—The contribution of amateurs to ornithological science has been tremendous. By means of this available man power ornithologists have been able to attack and solve problems quite beyond the present reach of investigators in other fields of biology. Nevertheless, there is a wide gap between the results that could be accomplished and those actually being obtained by this great body of bird students. This first American guide to bird study should do much toward directing the work of amateur bird students into useful channels.

The author wisely refers his readers to the work of James Fisher and Margaret Nice for information on the literature of bird watching and the study of bird behavior. His own book effectively treats such subjects as bird migration, population, distribution, banding of birds and methods of bird study. A very useful appendix includes an outline for life history study, a classified bibliography and a very full list of American bird clubs.

Every one interested in birds should have this very stimulating and instructive book.—J. VAN TYNE.

Archivos de Zoologia do Estado de São Paulo. Volume III (Tomo XXVI da Revista do Museu Paulista), 1942: i–vi, 1–849, illustr. **Papéis Avulsos do Departamento de Zoologia,** Secretaria da Agricultura, Indústria e Coméreio, Vol. II, 1942: i–iv, 1–336, illustr., and Vol. III, 1943: i–v, 1–336, illustr.—The Brazilian state of São Paulo has long been known as a center of zoological research. These three volumes, recently received, give abundant evidence that natural history continues to be actively prosecuted there. Several North American specialists join hands with their Brazilian colleagues in helping to make known the immense fauna of Brazil. The volume of the Archivos contains two large reviews, one by J. LANE AND N. L. CERQUEIRA on the mosquitoes of the tribe Sabethini and one by CARLOS O. DA CUNHA VIEIRA on the bats of Brazil. HENRY W. FOWLER contributes a bibliographic check list of the coastal fishes of that country. Entomology predominates, but many groups of animals are dealt with in the three volumes.

SHORTER ARTICLES AND DISCUSSION

AN EXPANSION OF JONES'S THEORY FOR THE EXPLANATION OF HETEROSIS

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JONES (1917) developed a theory for the explanation of heterosis which has been accepted by most geneticists and plant breeders. Briefly, this hypothesis is as follows: (1) a large number of genes are responsible for the differentiation of most quantitative characters and (2) those genes favorable to the production of the desirable quantitative character are at least partially dominant. The histories of the sciences reveal that, as more data covering a greater range of materials and environments are analyzed, it frequently becomes necessary to expand and modify theories for the explanation of scientific phenomena. Richey (1942) realized that certain characters do have a multiplicative effect on other characters and proposed that "mock dominance" be eliminated from further consideration in connection with hybrid vigor. Dempster (1943) discusses "mock dominance" further and concludes that though the existence of gene interaction based on certain measures might be relatively insignificant from the standpoint of practical application, it is doubtful whether it should be described as "mock dominance." The purpose of this article is to point out the need for and present an expansion of the theory for the explanation of heterosis.

Any discussion of heterosis of necessity involves a discussion of dominance. This latter term is used by geneticists in two quite different senses. (1) It has been used to denote the degree of expression of one or the other of the two contrasted characters resulting from the action of the heterozygous gene pair (Aa) plus the action of the environment, in which A represents any gene and a its allel. Then, an intra-allelic interaction of A and a may be involved as well as an interaction with the environment. In such studies the genotypes are known or determined, at least in respect to the major gene pair or pairs differentiating the contrasted characters under consideration. Also, the balance of the genotypic complex and the environment presumably have been

controlled sufficiently to allow the making of valid comparisons involving the heterozygote Aa and the two homozygotes AA and aa. It is evident that in such studies the material is not limited to the F_1 generation and the two parents, but may include any comparable material in which the heterozygote Aa and the two homozygotes AA and aa can be identified. (2) Also, dominance has been used to denote the degree of expression of one or the other of the two contrasted characters in the F_1 generation as compared to the expression of these contrasted characters in the two parents. The parents should approach homozygosity sufficiently close so that such comparisons are justified. Then, it is apparent that both intra-allelic and inter-allelic interactions may be involved as well as interactions between the genes and the environment. Obviously, under (2) the material is limited to the F_1 generation and the two parents. In this article the use and scope of the term dominance is that given under (1) & (2).

The theory of gene action as developed by Goldschmidt (1938) aids materially in understanding the phenomena of dominance and heterosis. That the genes control rates of reactions (Wright, 1916; Goldschmidt, 1917) is basic to this theory. Other biological phenomena used by Goldschmidt in developing his theory of gene action are thresholds, timing, interrelationships of the different reactions catalyzed by the genes and the numerical systems involved. For a discussion of these phenomena the reader is referred to Wright (1916, 1941) and Goldschmidt (1938). The numerical systems and their relationship to the nature of the interactions of the genes as measured by end products are discussed in more detail by Powers (1939, 1941). These physiological genetic theories basic to an understanding of dominance and heterosis will not be discussed in detail in this article but should be kept in mind while interpreting the data which follow.

To facilitate discussion, data are given in Table 1 for number of ripe fruit per plant, size of fruit and yield of ripe fruit per plant of certain F_1 tomato (*Lycopersicon*) hybrids and their parental inbred lines. An understanding of the relationship existing between the characters is essential to an interpretation of the data: Number of ripe fruit per plant and size of fruit are subcharacters of yield, as the product of number of ripe fruit by weight of fruit (size) gives yield. Any discrepancies noted in the table are due to dropping of decimals in calculating size of fruit.

The data in Table 1 show that the following situations exist as regards the subcharacters number of and size of fruit. The F_1 hybrid 4109 × 4110 shows heterosis for number of ripe fruit per plant and partial dominance for large size fruit. In the F_1 hybrid between 4101 and 4103 more fruits per plant is dominant and size of fruit shows no dominance. Finally, both few fruits and small size are partially dominant in the F_1 hybrid 4102 × 4110. Yet, in all three of these hybrids number of fruit and weight of fruit combine to produce heterosis for yield.

The third situation was found to exist for four other crosses; namely, when 4101 was crossed with 4109 and 4110 and when 4102

TABLE 1

NUMBER OF RIPE FRUIT, SIZE OF FRUIT AND YIELD OF RIPE FRUIT OF THREE F_1 TOMATO HYBRIDS AND THEIR PARENTAL INBRED LINES

Hybrid or inbred line	Ripe fruit	Size	Yield
	Number	Grams	Grams
4109	118.3 ± 12.91	12 ± .50	1364 ± 151
F_1 hybrid	183.2 ± 13.32	16 ± .68	2876 ± 177
4110	109.1 ± 11.34	17 ± .58	1868 ± 149
4101	4.3 ± .32	119 ± 6.49	513 ± 39
F_1 hybrid	20.5 ± 2.27	89 ± 3.66	1827 ± 196
4103	19.5 ± 2.94	55 ± 2.74	1066 ± 159
4102	4.4 ± .69	138 ± 12.81	607 ± 86
F_1 hybrid	44.5 ± 2.52	55 ± 2.93	2428 ± 150
4110	109.1 ± 11.34	17 ± .58	1868 ± 149

was hybridized with 4109 and 4110. Hence, these findings are well established statistically. Also, Powers (1941) in previous investigations found that fewer fruits per centimeter of branch exhibited either complete or partial dominance and that small size of fruit in crosses involving *Lycopersicon pimpinellifolium* (Jusl.) Mill. and *L. esculentum* Mill, was partially dominant. Then, that less desirable characters exhibiting partial dominance may in certain instances combine to produce a third highly desirable character which shows heterosis is well established.

The fact that partial dominance for the smaller of two contrasted quantitative characters occurs would lead one to suspect that the genetic phenomena responsible for dominance and heterosis would produce values less than that of either parent. Two such cases have been reported for mean number of fruit per centimeter of branch. Danmark × Johannisfeuer and Danmark × Red Currant were the crosses for which Powers (1941) found fewer fruits per centimeter of branch to exhibit heterosis.

The inbred lines whose F_1 hybrids (Table 1) showed partial dominance for small number of fruits per plant were selected from crosses involving the three parents, Danmark, Johannisfeuer and Red Currant. Hence, it is not surprising that partial dominance for fewer fruits should occur. The data on average number of locules per fruit for a hybrid involving two of these inbred lines are listed in Table 2.

TABLE 2

MEAN NUMBER OF LOCULES PER FRUIT

Hybrid	P_1	F_1 hybrid	P_2
4110, $P_1 \times$ 4101, P_2	9.6 ± .328	7.3 ± .249	11.7 ± .499

The F_1 hybrid has fewer locules per fruit than the fewer loculed parent 4110. The same was true of six other hybrids from a total of nine which had 4110 as one of the parents. Hence, that this phenomenon does occur is well established experimentally.

Clearly, an expansion of Jones's theory for the explanation of heterosis is desirable. His theory as presented does not take into account the fact that in some cases the effects of the genes are geometrically cumulative. The first part of Jones's hypothesis, namely (1) a large number of genes are responsible for the differentiation of most quantitative characters still holds. The second part, (2) those genes favorable to the production of the desirable quantitative character are at least partially dominant needs to be expanded; because in the cases in which the effects of the genes are multiplicative, those genes not favorable to an increase of the quantitative character may show no dominance or partial dominance and still the character itself may show beneficial heterosis. This statement may be simply illustrated as follows. Assume that number of fruit for the inbred lines 4102 and 4110 are differentiated by an allelic pair of genes AA and aa; and likewise assume that size of fruit is differentiated by an allelic pair of genes BB and bb. Then the genotypes of the parents and F_1 hybrid of Table 1 for the cross 4102 $\times$ 4110 would be:

4102	AAbb
F_1 hybrid	AaBb
4110	aaBB

It is evident that the heterosis of the F_1 hybrid for yield (2,428 grams) would be due to the multiplication of the effects of Aa (44.5) by the effects of Bb (55); yet, both Aa and Bb are partially

dominant for lower yield. The genotypic situation depicted is probably far simpler than that which actually exists, but it does serve to illustrate that the assumption of partial dominance of those genes favorable to the production of the quantitative character is not necessary when the inter-allelic interactions of the genes are such that their effects are multiplicative. In fact, there may be no dominance or even partial dominance of those genes not favorable to an increase of the quantitative character under consideration and still the character could show heterosis.

In order to understand heterosis, it in necessary to realize that this phenomenon is a phase of quantitative inheritance, the same as are dominance and partial dominance. In reality, as previously pointed out by Powers (1941), heterosis and dominance are different degrees of expression of the same physiological genetic phenomena. This is simply illustrated by the graph of Fig. 1. On the graph the position of the F_1 hybrid determines the type

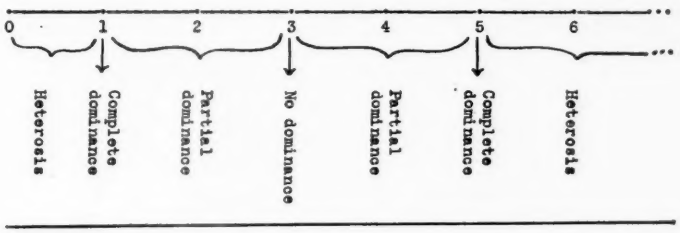

FIG. 1. Values of F_1 Hybrids and Parents in Relation to Heterosis and Dominance.

of heterosis or dominance that is exhibited. For example, if the value of the F_1 hybrid falls between 3 and 5, partial dominance for an increase of the quantitative character would be exhibited. That the expression of dominance is dependent upon both the genotype and environment has been demonstrated by a number of workers (Goldschmidt, 1938); and that dominance may be shifted to heterosis or vice versa, by varying either the genotype or environment, has been shown by Powers (1941). Hence, it is evident, as illustrated by the graph, that heterosis and dominance are different degrees of expression of the same genetic phenomena and are dependent upon both the genotype and environment.

Before closing the discussion, a matter of terminology should be mentioned. Many plant breeders and geneticists have become

accustomed to associating heterosis with the beneficial or more desirable character. There seems to be no genetic reason for making such a distinction. However, in plant breeding sometimes a distinction is desirable. Therefore, rather than coin any new terms to fit the genetic interpretation given here, it is suggested that the term heterosis be employed in the usual sense and non-beneficial heterosis be employed when the F_1 hybrid exhibits heterosis for the less desirable of the two contrasted characters.

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TRANSPLANTATION AS A TOOL OF DEVELOPMENTAL GENETICS

THE transplantation technique has been of inestimable value in many fields of experimental biology, especially in the analysis of embryonic induction and in the study of endocrine relationships. In the rapidly growing science of physiological genetics there will undoubtedly be many problems which can be approached by transplantation methods. In fact, some important advances of this kind have already been made. There is sufficient reason, then, to call attention to limitations inherent in these methods and which must not be lost from sight if confusion is to be avoided.

The ultimate question of interest to the physiological geneticist is, of course, that of gene action. In this respect we can not look for unequivocal information from transplantations. For, even in the relatively simple cases in which traits are determined by diffusible substances and even if the production of these sub-

stances appears to be limited to certain parts of the organism, no satisfactory solution of our problem is afforded by the assumption that the elaboration of the diffusible substance is virtually identical with the activity of the gene in question.

If we except the apparently rare occurrence of the elimination of definite chromosomes in the developmental history of certain organs, it must still be assumed, any evidence to the contrary lacking, that all genes are distributed to all cells of the body. The problems of differentiation will not be solved by the *ad hoc* assumption that genes have been lost from certain parts. All biological experience speaks against the justification or general validity of such an hypothesis. There is a definite possibility, to be sure, that cytoplasmic differentiation curbs or modifies the activity of certain genes in specific parts, but we have at present no factual evidence whatever in support of this assumption.

Even if the experimenter's question relates only to the problem of the determination of certain hereditary features within a single organ the transplantation technique can not always be expected to give a definite answer. This is to be illustrated here with reference to recent experiments by Gayer and Hamburger (1943) on material of Creeper fowl. In earlier experiments Gayer (1942) had demonstrated that eye anlagan of genetically normal chicken embryos, when transplanted to the flank of genetically normal hosts, generally show in later stages abnormalities which are very similar to those typical of intact late homozygous Creeper embryos. It was clear, therefore, that "phenocopies" of all essential features of homozygous Creeper eyes could be produced by the simple expedient of transplantation to an unusual locality. In their new and beautifully executed experiments Gayer and Hamburger (1943) were able to show that the orthotopic transplantation of normal eye anlagen (including surrounding tissues) to normal hosts leads in most cases to the development of entirely normal eyes.

On the basis of these new observations Gayer and Hamburger (1943) set out to find an answer to the following alternative questions: "Does the Creeper factor, in doube dose, in producing its effects on the eye, act locally, starting in the cells of the eye primordium itself; or indirectly, starting in structures outside of the eye, and transmitting its effects by way of inductions or similar mechanisms?" The fact that normal eye anlagen transplanted orthotopically develop into normal eyes was assumed to provide the basis for "crucial" tests.

What were the results? (1) Many transplants of homozygous Creeper (CpCp) primordia developed entirely normally within genetically normal (cpcp) hosts. This, the authors admit, "gives no crucial answer." For, "it can be explained either by assuming that the transplant was deficient, and the deficiency was remedied by the cpcp milieu ——, or by assuming that the transplants had no intrinsic deficiencies at all." (2) Transplants of CpCp eye primordia to cpcp flanks in subsequent development produce the typical features of homozygous Creeper embryos. This result is again, and rightly, dismissed by Gayer and Hamburger (1943) as "not crucial." For, "one may argue that the flank region of the cpcp host, in contrast to the head region, does not provide for an adequate supplementary agent, at least not in the necessary quantity." (3) A single instance is recorded in which a homozygous Creeper embryo bearing an orthotopic transplant of a normal embryo survived sufficiently long to diagnose the condition of the eye in the transplant. The eye showed abnormalities similar to those of the host, but less extreme. The following features are worthy of note. "The transplant eye is larger than the host eye." The coloboma of the transplant eye is "of lesser extent," "the retina duplication only along one margin of the choroid fissure," and there was in certain parts of the sclera an "abnormally thick cartilage layer." Gayer and Hamburger (1943) think this case tends to confirm the interpretation that the typical coloboma of homozygous Creeper eyes "is imposed on a potentially normal eye by factors located in the phocomelic head." They are forced to admit, however, that "the validity of this case is weakened by the following considerations. The host head as a whole is abnormal and misshapen, and it is conceivable that the eye abnormalities are due to this condition," and they call attention to the fact that the same abnormalities had been produced by Gayer (1942) in genetically normal eyes by transplanting them to an abnormal environment, *viz.*, to the flank. In our opinion, it would be impossible to draw valid conclusions even if further experiments of the same type should yield similar results, and even if such results were obtained in the absence of other head deformities; for it is difficult to see why the argument which Gayer and Hamburger (1943) used in the interpretation of their results with transplants of CpCp eye primordia to cpcp flanks (quoted above) should not apply to this situation as well, or why, in fact, it should not apply to the results of transplanting normal primordia to the flank of normal hosts.

Such are, briefly, the results of the experiments. The conclusions which the authors draw are definite and unequivocal: "(a) The deficiencies are not due to 'local gene action' in the eye primordia. The Cp-factor, in producing these deficiencies, operates in an indirect way. The primary action is on structures extrinsic to the eye. (b) Whereas CpCp embryos are non-viable, CpCp eye primordia not only survive in orthotopic position but they show no effect of the CpCp-genotype which they carry in each of their nuclei."

To us, on the other hand, it seems that all the results can readily be interpreted in quite another fashion. Let us make the following assumptions: (a) a substance x is normally elaborated by all embryonic tissues and cells or by certain types of cells occurring throughout all tissues; (b) the elaboration of substance x is hindered by abnormalities in the supply of oxygen or certain nutrients; (c) substance x is needed for cell multiplication and/or certain steps of differentiation; (d) substance x can, if necessary, be taken up by cells from the blood or from neighboring tissues; (e) the rate of production of substance x is reduced in the presence of the Creeper mutation.

These are scarcely unusual assumptions or ones which are unlikely to occur. If such conditions should actually prevail, what results could be expected in the various situations which underlie the experiments of Gayer and Hamburger (1943)? The eye abnormalities of late homozygous Creeper embryos would, of course, be expected by definition. In the case of cpcp transplants to the flank subnormal conditions of circulation might be anticipated to result in the phenocopies which were found. Either the same argument or the intrinsic conditions of their Creeper nature would apply to CpCp transplants to the flank of cpcp embryos. In cpcp eye primordia orthotopically transplanted to CpCp embryos it might again be the unfavorable conditions after transplantation which prevent the anlage material from producing a normal eye. On the other hand, either cpcp or CpCp eye primordia transplanted orthotopically to cpcp heads might develop normally on account of sufficient supplementation reaching the transplant in this location and, in the case of CpCp transplants, thereby overcoming intrinsic deficiencies. If these arguments hold for the morphological eye abnormalities (coloboma, etc.), they can be applied *a fortiori* to growth of the eye.

It is not our intention to assert that the assumptions which

have here been made actually exist under the conditions of the experiments which have been discussed. But we do wish to point out that the transplantation experiments of Gayer and Hamburger (1943) are quite incapable of differentiating between conditions, such as would exist under our assumptions and the developmental situation actually postulated by them on the basis of their results.

Development of the normal eye may be referred to as "non-autonomous," if judged by the fate of heterotopic transplants; it may be looked at as "autonomous" in view of the results of orthotopic transplantation. The two designations are descriptive of each particular set of circumstances rather than explanatory of underlying causes. It is similar with the eyes of homozygous Creeper embryos. To put it into cruder terminology, the fact that a "diseased" part remains abnormal in a "diseased" organism, but becomes healthy in a favorable environment neither proves nor disproves that the part in question was not, to start with, in a pathological condition.

The conclusions at which Gayer and Hamburger (1943) arrived, as far as reference has been made to them by us, constitute one possible explanation of the established facts. They are no more than a hypothesis, however, and evidence of a different nature will be needed to verify whether or not these particular suppositions represent the correct explanation.

Situations similar to the one which has been discussed here will undoubtedly arise often in the developmental analysis of genetic traits. It was our purpose to show that transplantation experiments will not always be capable of providing conclusive information and that, in any event, great circumspection must be exercised in the interpretation of their results. Such caution will probably be particularly necessary whenever in the analysis of hereditary variations there is a possibility that partial or complete suppression in the elaboration of diffusible substances or alterations of their chemistry may play a role.

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SELF-FERTILIZATION IN THE RUSSIAN DANDELION, *TARAXACUM KOK-SAGHYZ*

THE Russian dandelion normally is self-sterile and sets seed sparingly, if at all, when protected against cross-pollination by insects.[1,2,3,4] During the past year and one half, an opportunity was provided for further observations of self-fertility and self-sterility in this species, on greenhouse plants being grown for studies in cytology and rubber content.[5] These observations indicate a wide variability in extent of flowering and self-fertility, apparently associated with the season.

Seeds were sown in seed pans on May 16, 1942. A month later, between 800 and 900 seedlings were transplanted into 3-inch pots; these were later transferred to 4-inch and 5-inch pots as required. All plants were given liquid organic fertilizer from time to time and thus kept in a growing condition, although many became pot-bound before the end of the study. The greenhouse was heated during the winter, and a night temperature of between 55° and 65° F. and a day temperature of between 65° and 75° F. was maintained. Summer temperatures in the greenhouse were generally somewhat higher than those prevailing out of doors. No artificial illumination of any sort was given. The greenhouse was screened, and thus was free from insects.

On August 16 the first plant came into flower, and others followed intermittently throughout August, September and October. Of the hundreds of blossoms produced by a score or more of plants which flowered during August and September, only one head, containing four seeds, was set. Numerous hand-pollinations were made by rubbing together two blossoms from the same plant. None of these produced seed; although similar pollinations between two different plants produced the normal set of 40 to 80 seeds. *T. kok-saghyz* is obviously highly self-sterile under these conditions.

[1] V. Poddubnaja-Arnoldi and V. Dianowa, *Planta*, 23: 19, 1934.

[2] V. A. Koroleva, *Soviet Plant Ind. Record*, 2: 1, 1940. (Translation No. 21, U.S.D.A. Rubber Plant Investigations.)

[3] H. E. Warmke, *Bull. Torrey Bot. Club*, 70: 164, 1943.

[4] H. A. Borthwick, M. W. Parker and N. J. Scully, *Bot. Gaz.*, 105: 100, 1943.

[5] This work was undertaken in cooperation with the Rubber Plant Investigations Project of the Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Department of Agriculture.

Further observations on flowering and seed setting were arbitrarily limited to a group of 382 representative plants. The data collected from this group of plants are shown graphically in Fig. 1. A total of 159 plants, or nearly 42 per cent. of the total, flowered between the middle of August and the end of the following May. Two maxima are evident: one in November, when 66 plants were observed to flower, and another in April, when 50 plants came into flower. These maxima are separate and distinct,

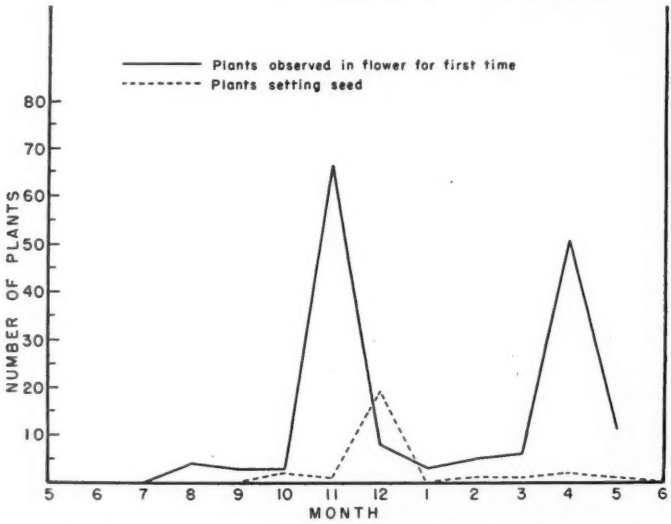

FIG. 1. Flowering and seed record of 382 greenhouse plants of *T. koksaghyz*. Three maxima are evident: two in flowering (November and April) and one in the number of plants setting seed by self-fertilization (December).

because during the latter part of December and all of January and February few new plants came into flower and those that had flowered earlier returned to a vegetative state. This would seem to indicate an inhibition of the flowering response during the middle of the winter, even though conditions were such that vegetative growth continued.

Flowering records were discontinued on June 1, but seed records were continued until July 1, when the plants were transplanted out of doors and all record taking was discontinued. The rapid decline in new flowering during May, however, probably

indicates the onset of a summer dormancy, previously observed in this species by others workers.[6, 7]

The broken line in Fig. 1 records the number of plants from which mature seeds were harvested during the course of the observations. A pronounced peak in seed setting is observed in December, following the outbreak of flowering in November. No such peak is observed following the April flowering maximum. During October, November and December seeds were collected from 22 (26.2 per cent.) of the 84 plants which had flowered up to that time. This is in sharp contrast to the five plants (6.7 per cent.) which set seed among the 75 plants that came into flower between January 1 and the end of May. The yield of seed, also, was greater in the winter than in the spring. The 22 fall- and winter-flowering plants from which seed was harvested set 53 heads with a total of 1,690 seeds, or an average of 31.9 seeds per head and 76.8 seeds per plant. The five plants which set seed during the late winter and spring produced six heads with a total of 110 seeds, or an average of 18.3 seeds per head and 22 seeds per plant.

The causes for the flowering maxima in November and April and for the high percentage of seed set in December under the above conditions are not clear. Since the experiment was not planned as a study in temperature and photoperiod, no conclusions are legitimate beyond the simple observations that the flowering maxima occur at approximately the onset and the end of the cold season, both on relatively short photoperiods (one decreasing and the other increasing), and that the high set of seed in December may possibly be a sort of "end-season fertility," a phenomenon commonly observed in *Nicotiana*[8] and other self-sterile species. Borthwick, Parker and Scully,[9] in a series of controlled experiments, have indicated the importance of low temperature in the induction of flowering in this species.

Since the development of an unreduced and unfertilized egg is characteristic of the polyploid members of the genus, it seemed

[6] V. A. Koroleva, *Soviet Plant Ind. Record*, 2: 1, 1940. (Translation No. 21, U.S.D.A. Rubber Plant Investigations.)

[7] G B. Neiman and A. A. Sosnovets, *Dok. Veses. Akad. S. Kh. Nauk im Lenina*, No. 2: 15, 1941. (Translation No. 703, U.S.D.A. Soil Conservation Service.)

[8] E. M. East and J. B. Park, *Genetics*, 2: 505, 1917.

[9] H. A. Borthwick, M. W. Parker and N. J. Scully, *Bot. Gaz.*, 105: 100, 1943.

possible that this rather abundant seed setting in the absence of insects might have been apomictic—in spite of the fact that *T. kok-saghyz* has been shown normally to reproduce in a sexual manner.[10,11] To determine the nature of the process by which the seeds were produced, a study of the characteristics of the offspring was made. Since the original Russian stock was highly heterozygous, as indicated by wide variation in habit and leaf characters, the offspring of any given plant should show genetic segregation if they result from sexual self-fertilization. If the offspring are the result of apomixis, they should be much more uniform in appearance.

Four hundred and sixty-three offspring were obtained from the seed of uncrossed heads of 15 different plants. These were observed until they were nine months of age, at which time many had flowered. The variability among the offspring from a given plant was nearly as great as in the original Russian stock: some leaves were large, others were small; some leaves had entire or nearly entire margins, while others were deeply incised; some had considerable color in the midrib, while others were colorless. Such extensive variation can hardly be thought of as the result of environmental influences; it points strongly to genetic segregation and thus to true sexual self-fertilization.

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[10] V. Poddubnaja-Arnoldi and V. Dianowa, *Planta*, 23: 19, 1934.
[11] H. E. Warmke, *Bull. Torrey Bot. Club*, 70: 164, 1943.